



PGIS POSTGRADUATE RESEARCH HIGHLIGHTS 2016

**Research Highlights of the Postgraduate Institute of Science (PGIS)
University of Peradeniya**

**PhD
RESEARCH**

**MPhil
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COVER

Playing with light in the deep blue

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POSTGRADUATE INSTITUTE OF SCIENCE

**UNIVERSITY OF PERADENIYA
SRI LANKA**



**PGIS POSTGRADUATE
RESEARCH HIGHLIGHTS 2016**

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PGIS Postgraduate Research Highlights 2016

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MESSAGE FROM THE DIRECTOR

Research is the key to the success of a higher educational institute, which in turn would contribute to industrial development and economic growth of the country. Over the years, it has been concentrating on research activities, in both fundamental and applied sciences, in the disciplines of Biochemistry & Molecular Biology, Chemistry, Earth Science, Environmental Science, Mathematics, Physics, Plant Science, Science Education, Statistics & Computer Science and Zoology.

With the progressive expansion of the PGIS, research laboratories in specialized areas of Computer Science, GIS & Remote Sensing, Instrumentation and Soil & Rock Mechanics have been established within the institute. The research activities of the PGIS students are conducted, mainly at the Faculty of Science of the University of Peradeniya, and at other research institutions, such as the National Institute of Fundamental Studies.

In order to disseminate novel research findings of national relevance and significance effectively, and to encourage young researchers who would shape the future of the country in the field of research and development, this year, the PGIS took another step forward. This is by publishing the research findings of postgraduate research students in a less technical form. The launch of the publication on 'PGIS Postgraduate Research Highlights', on 07th October 2016, the day of the 20th Anniversary Celebrations, will mark another milestone of the PGIS. This publication includes research findings of MPhil and PhD candidates who were awarded postgraduate degrees at the General Convocation 2015 of the University of Peradeniya. The publication 'PGIS Postgraduate Research Highlights' would be an annual publication of the PGIS, from this year onwards.

I appreciate the dedication and untiring efforts of the Editorial Team, headed by Prof. Jayanthi Edirisinghe, Emeritus Professor of the University of Peradeniya, to make this valuable exercise a success. I am also thankful to the MPhil and PhD holders of the PGIS, as well as to their supervisors, for contributing to this publication.

Prof. HMD Namal Priyantha
Director, Postgraduate Institute of Science
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MESSAGE FROM THE EDITOR IN CHIEF

The PGIS inaugurated the Annual Research Congress in 2013 providing a platform to researchers in all disciplines of Science, enabling them to present their research findings. The third of this series is being held in 2016.

This year, yet another milestone has been added to the history of the PGIS through the publication of the "PGIS Postgraduate Research Highlights 2016". The idea of such a publication was conceived in order to showcase the output of all postgraduate research of PGIS registrants, leading to higher research degrees, in a given year.

This publication provides a platform for PGIS researchers who received their PhD and MPhil Degrees at the General Convocation 2015, to disseminate their findings in print form, to the rest of the scientific community. A total of 25 graduands of the PGIS received their postgraduate degrees at the General Convocation 2015 of the University of Peradeniya, held on 26th May 2016. Among them are 14 PhD Degree recipients and 11 MPhil Degree recipients. PGIS Postgraduate Research Highlights 2016 include research of 12 PhD recipients and 9 MPhil recipients. A brief summary of the research study in a somewhat non-technical manner together with a short profile and a photograph of the researcher are provided under the respective thesis titles. The contents are arranged alphabetically according to the surname of the degree recipient starting with the PhD Degree recipients, as listed in the General Convocation 2015 Handbook. PGIS Postgraduate Research Highlights 2016 will be launched at the 20th Anniversary Celebrations of the PGIS on the 7th of October 2016.

I take this opportunity to thank the recipients of postgraduate research degrees and their supervisors for providing the necessary information to make this publication a reality. To the Editorial Committee who worked tirelessly to bring out this pioneering issue in a short period of time and to Prof. H.M.D. Namal Priyantha, Director PGIS, who supported this idea to the end, I owe a big thank you.

Prof. Jayanthi P. Edirisinghe
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Identification of the genomic regions associated with phosphorus deficiency tolerance in Sri Lankan rice germplasm for marker assisted breeding



W. B.W. M. R.Y. Chathurika Aluwihare graduated in 2008 from the University of Peradeniya with a BSc Degree in Biological Science and immediately embarked on a Masters' programme in Molecular Life Sciences at the University of Colombo. Thereafter, Chathurika joined the research staff of the Department of Agriculture at Mahailuppallama. She is trained in Molecular Plant Breeding at the International Rice Research and Development Institute (IRRI), Philippines. Chathurika obtained her PhD Degree in 2015 from the PGIS. **Email:** yasminaluwihare@gmail.com

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Rice is the staple food of all Sri Lankans and rice cultivation is the livelihood of about 1.8 million farmer families engaged in the production of rice. Profitable rice farming is essential to provide rice at a reasonable price and to ensure a steady income to the farmers and other stake holders. Modern rice cultivars require application of fertilizer to achieve higher productivity. As such, phosphorous (P) fertilizers, such as triple super phosphate (TSP) and rock phosphate, are routinely used by rice farmers. Application P fertilizer has become mandatory, as rice growing soils lack available P, due to P fixation with Al^{3+} and Fe^{3+} ions in the soil. As a result, farmers tend to apply more and more P fertilizer in rice farming. The Sri Lankan government spends annually, nearly Rs. 1200 million on P fertilizers that amounts to 0.2% of the GDP of the country. Application of P fertilizer contributes to environmental pollution and health problems, as well. Hence, there is a strong demand for organically grown rice which depends on organic manure such as, crop stubbles that lack sufficient P levels for plant growth and productivity.

Sri Lanka, so far, has not focused on breeding of P deficiency (PD) tolerant rice cultivars. Internationally, PD tolerance in rice has been studied to a great extent. Researchers elsewhere have identified a major quantitative trait locus (QTL), *Pup1* for PD tolerance, fine mapped, bioinformatically analyzed and molecular markers have been developed. These markers could be used in marker assisted breeding (MAB). However, these studies have used a japonica rice cultivar, *Nipponbare* and an Indonesian land race *Kasalath*. In Sri Lanka, only genotyping of a set of rice cultivars just with two DNA markers has been carried out. Internationally characterized QTLs such as *Pup1* cannot be directly used in MAB of rice in Sri Lanka due to differential effects of genetic backgrounds, possible epistatic and environmental effects. Therefore, validation of *Pup1* haplotypes with respect to the degree of PD tolerance in Sri Lankan rice germplasm is essential to initiate a MAB program for PD tolerance in rice.

This study strived to achieve several objectives. Firstly, to screen mega production varieties and important landraces of rice for PD tolerance, to establish an objective indexing system to characterize any rice variety or a genotype for PD tolerance, using soil based screening procedure. Secondly, to identify the DNA marker haplotypes of *Pup1* QTL using DNA markers of the screened cultivars and to design effective and efficient markers that can discriminate *Kasalath* and *Nipponbare* like *Pup1* haplotypes. This would lay the platform for marker assisted selection (MAS) towards PD tolerant rice varieties in Sri Lanka. Thirdly, to identify the polymorphism of *Pup1* QTL haplotypes by DNA sequencing and bioinformatic analysis, using a codominant locus and to detect the associations between SNPs and traits with respect to PD tolerance. This experiment would reveal any novel QTL haplotypes based on the SNPs and provide a deep insight as how to sequence polymorphism in MAS for PD tolerance. The next objective was to generate F₂ population using PD tolerant and PD sensitive cultivars as the parents to study the genetics and trait distribution in relation to PD tolerance and to conduct bulk segregation analysis (BSA), validate molecular markers and to characterize the genomic DNA in *Pup1* QTL of PD tolerant rice cultivars. Thereafter, establish the molecular basis for selection of the highest tolerant genotypes from rest of the cultivars. The final objective was to validate the PD tolerant traits using additional F₂ segregating progenies and to develop a statistical software to check the goodness

of fit ratio of traits associated with PD tolerance.

A set of 30 rice landraces and varieties was screened for PD tolerance and the following parameters: plant height, number of tillers, shoot dry weight, shoot P concentration, shoot P uptake and P use efficiency were measured and statistically analyzed. A scoring system was developed to express the PD tolerance in rice varieties; 3 - tolerant, 2 - moderately tolerant and 1 - sensitive.

Haplotype analysis was conducted for 23 DNA markers, 30 rice genotypes and the reference PD tolerant landrace *Kasalath* and PD sensitive landrace *Nipponbare*. The DNA sequence analyses were performed for K20 and K46 loci within the *Pup1* region. The genetic analysis based on segregating populations and bulk segregant analysis (BSA) were adopted to validate the DNA markers for MAS.

A total of 30 rice cultivars were screened for phosphorous deficiency (PD) tolerance (PDT) during 2012 *Yala* and *Maha* seasons, under greenhouse and field conditions. An indexing system was developed to score the rice cultivars for PD tolerance (Table 1).

A total of 84 F₁ crosses were made among the specific parents, advanced to F₂ level for progeny analysis. These segregating populations are currently advancing to F₁₀ populations after which suitable recombinant inbred lines (RILs) would be selected for further analysis. The genetics and nature of trait

Table 1. The proposed scoring scheme for PDT screening of rice genotypes

3-Highest tolerant*	2-Moderately tolerant*	1-Sensitive*
H-4#	Pokkali	Bg 357\$
Marss	Bg 358	At 306
Suduheenati	Bg 450	At 354
H-10	Sudurusamba	Bg 300
Rathel	Bg 379-2	
Kaluheenati	Hondarawala	
Murungakayan	Bg 352	
Kokuwellai	Bg 250	
H-7	Bw 364	
Sudubalawee	At 353	
Bg 94-1	Suwandel	
Bg 403	Ld 356	
At 362	Rathuheenati	

*Rice genotypes listed in the order of decreasing PDT. #Highest tolerant genotype.

\$Highest sensitive genotype

distribution in relation to PDT was confirmed by H4 × Bg 357, *Marss* × Bg 357 and *Murungakayan* × Bg 357.

Sensitive and tolerant progeny individuals were selected and subjected to Bulk segregation analysis (BSA). Initially, the *Pup1* linkage polymorphic markers were identified for the parents. The marker alleles that can be used for marker assisted selection (MAS) for PDT were identified. The genetics and nature of trait distribution were confirmed by using four more F_2 populations. The *Pup1* marker haplotypes of the 30 rice cultivars were identified. DNA sequencing of a codominant region and dominant region within *Pup1* region revealed the unique genetic diversity that confers PDT in the Sri Lankan rice germplasm. Molecular markers and their haplotypes were clearly defined for

the Marker Assisted Breeding of PDT rice varieties for Sri Lanka. The molecular marker haplotypes were clearly defined and tabulated for MAS for PDT (Fig. 1).

According to the findings, the improved genotype H-4 is the highest PD tolerant variety. Haplotype analysis revealed 31 haplotypes indicating very high diversity within *Pup1* region. The sequence analysis of codominant region *K20* revealed very high level of sequence polymorphism and five clusters where unique haplotypes were detected in the highest tolerant H-4, *Murungakayan* and H-10 within the Sri Lankan rice germplasm, which are different from *Kasalath* group. The genetic analysis based on the segregating population revealed that most of the traits follow quantitative inheritance and associated

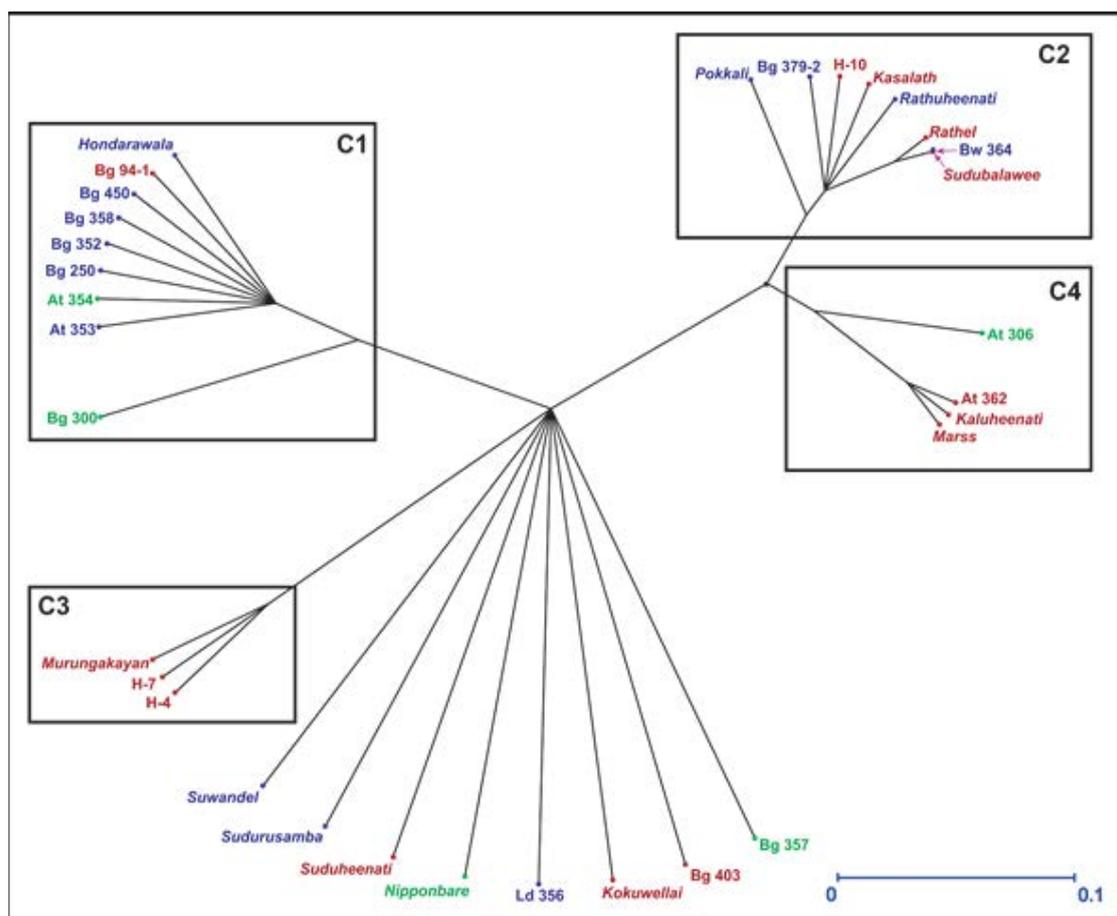


Figure 1. The Neighbour Joining Tree constructed for 32 rice cultivars based on the dissimilarity values derived from Simple Matching Coefficients calculated using *Pup1* linked DNA marker polymorphism. The algorithm Unweighted Pair Group Method with Arithmetic Means (UPGMA) in DARwin Software Version 6.0 was used for the construction of tree. The lines are drawn according to the scale of genetic distance and the scale bar represents 0.1 of genetic dissimilarity. Brown colour fonts indicate the PD tolerant rice cultivars, blue colour fonts indicate moderately PD tolerant rice cultivars and green colour fonts indicate the PD sensitive rice cultivars. *Kasalath*, standard PD tolerant and *Nipponbare*, standard PD sensitive cultivars are used as reference rice genotypes.

colour changes due to P starvation, epistatic dihybrid ratios, very high heterosis and heritability, indicating the higher chance of mapping QTLs effectively. The bulk segregant analysis (BSA) revealed the potential of applying marker alleles for marker assisted selection. The DNA marker analysis within the *K46* region provides the background to detect the highest tolerant cultivars separately from the tolerant cultivars for PD. The results obtained from the present study would undoubtedly contribute to the improvement of rice production with minimal use of P fertilizer.

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Thesis Reference

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Development of Sri Lankan vein graphite and modification of $\text{Li}(\text{Ni}_{1/3}\text{Mn}_{1/3}\text{Co}_{1/3})\text{O}_2$ as electrode materials for lithium-ion rechargeable batteries



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Finding new techniques to store more energy is currently an important area of research to address the challenges of increasing energy needs in the present technological era. Rechargeable Lithium Ion Battery (LIB) has emerged as a very promising rechargeable energy storage device. Current LIBs are based on expensive electrode materials, such as synthetic graphite as the anode material and lithium cobalt oxides as the cathode material. However, the high unit cost and unsolved materials-related problems are preventing the LIBs from reaching the people as a cheaper and reliable portable source of power. This study was undertaken to develop low-cost and performance enhanced electrode materials for rechargeable lithium-ion batteries (LIBs). The study was carried out by developing Sri Lankan natural vein graphite and modifying $\text{Li}(\text{Ni}_{1/3}\text{Co}_{1/3}\text{Mn}_{1/3})\text{O}_2$ based transition metal oxides. The following specific objectives were addressed in order to achieve this goal.

- i. Develop an effective low-cost method to purify natural vein graphite and for its surface modification.
- ii. Synthesize $\text{Li}(\text{Ni}_{1/3}\text{Mn}_{1/3}\text{Co}_{1/3})\text{O}_2$ and novel lithium transition metal oxides in the form of Li-Mn-Co-Ni-M oxides, where M is a cheaper metal additive, such as Mg, Na, Ba, and Cu.
- iii. Study the feasibility of using electrode materials developed in practical lithium ion batteries.

The purification of vein graphite to obtain 99.99% carbon was carried out using the Acid Leaching Technique.

For the surface modification of purified vein graphite, Mild Oxidation and Lithium Carbonate coating methods were used. For the development of the cathode, pure $\text{Li}(\text{Ni}_{1/3}\text{Mn}_{1/3}\text{Co}_{1/3})\text{O}_2$ and its derivatives were synthesized by Glycine Nitrate Combustion (GNC) process. Calcining was carried out at 900 °C for 2 h. Surface modification of purified graphite, phase analysis, particle characterization and electrical characterization of the electrode materials developed, were carried out by Fourier transform infrared (FTIR) spectra, X-ray diffraction

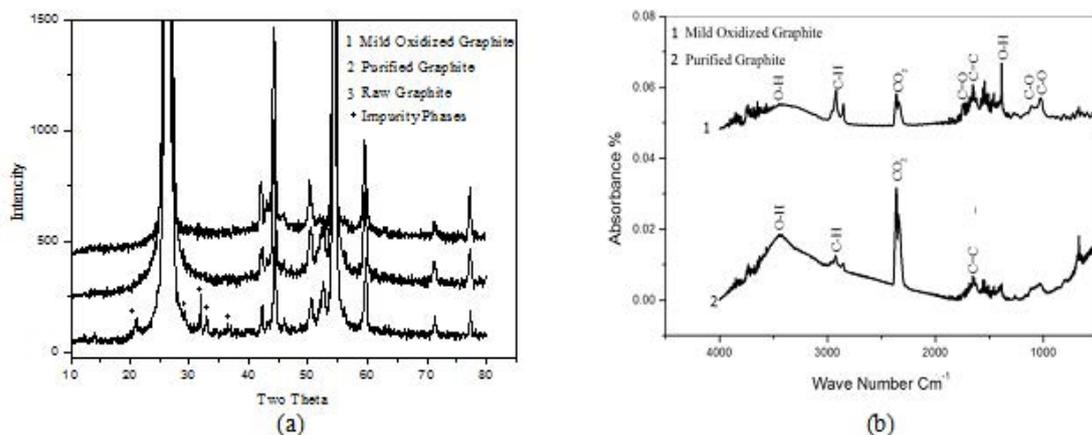


Figure 1. (a) XRD pattern and (b) FTIR spectrum of raw graphite (RG), purified graphite (PG) and mild oxidized graphite after purification.

(XRD), Scanning Electron Microscopy (SEM) and d.c. four probe technique. Electrodes were prepared by manual tape casting of electrode slurry (Active material + Binder + Conductive Additive + Solvent) onto respective current collectors by the Doctor Blade Method. Lithium ion coin cells were assembled using fabricated electrodes in an argon-filled glove-box with a porous polypropylene separator in a non-aqueous electrolyte, 1 M LiPF₆ in ethylene carbonate-diethyl carbonate-dimethyl carbonate (1:1:1 ratio in weight). The charge-discharge studies were carried out at C/5 rate with a cut-off voltage of 2.5 - 4.6 V for cathode materials and with a cut-off voltage of 1.5 - 0.002 V for anode.

The cost effective acid leaching method developed in this study showed successful removal of undesirable sulfide and carbonate impurities at very low acid concentration and at low temperature (Figure 1a).

Mild oxidation and lithium carbonate coating methods were investigated to improve the surface structure of the purified graphite. Chemical oxidation modified the graphite surface by formation of oxidizing species and elimination of structural imperfections (Figure 1b). The coating of graphite surface by Li₂CO₃ did not affect the crystal structure. The anodes fabricated with purified vein graphite showed promising discharge capacity but with poor cycle performance. However, the cycle performance of purified graphite was enhanced by both surface modification methods. The graphite electrode developed in this study is a promising anode due to its better electrochemical performance without expensive conductive additives.

For the development of the cathode, pure Li(Ni_{1/3}Mn_{1/3}Co_{1/3})O₂ and its derivatives were synthesized by Glycine Nitrate Combustion process,

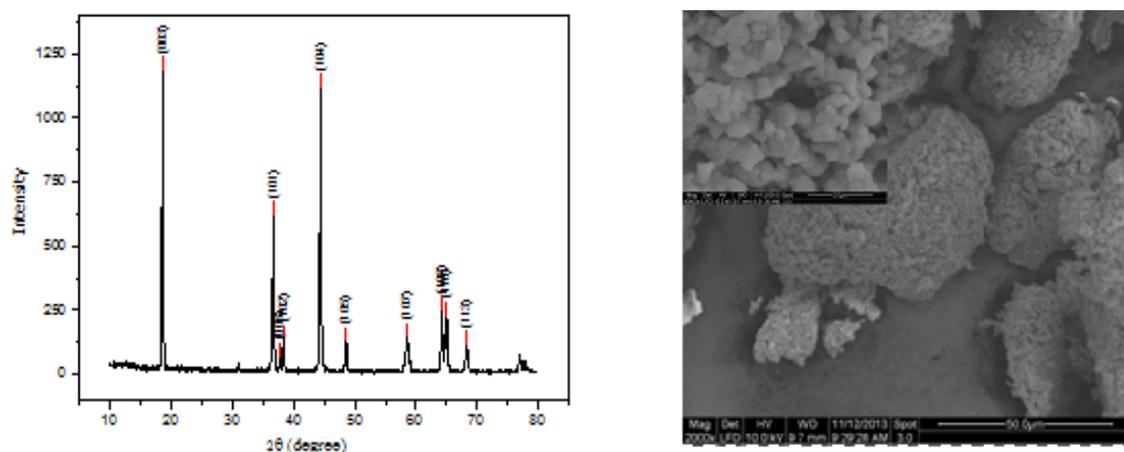


Figure 2. XRD pattern and SEM image of Li(Ni_{1/3}Co_{1/3}Mn_{1/3})O₂ prepared during this study.

while keeping the glycine:nitrate ratio at 1.0:0.6. All the synthesized powder compositions comprised sub-micron size secondary particles formed by aggregating nano-scale primary particles (Fig. 2). Electrical conductivity of all synthesized materials showed semiconducting behavior and improved conductivity in Na, Mg, Ba and Cu-Mg substituted compositions. The $\text{Li}(\text{Ni}_{1/3}\text{Mn}_{1/3}\text{Co}_{1/3})\text{O}_2$ showed the highest initial discharge capacity with significantly high rate capability. Overall, the study showed an improvement in electrochemical performance depending on the nature and level of substituting element, and its effect on cation mixing, enhancement of structural stability and electrical conductivity.

Of the synthesized materials, $\text{Li}(\text{Ni}_{1/3}\text{Mn}_{1/3}\text{Co}_{1/3-0.04}\text{Na}_{0.04})\text{O}_2$ showed an initial discharge capacity similar to that of $\text{Li}(\text{Ni}_{1/3}\text{Co}_{1/3}\text{Mn}_{1/3})\text{O}_2$ with an improved cycle performance. Cu-Mg co-substituted, $\text{Li}(\text{Ni}_{1/3}\text{Mn}_{1/3}\text{Co}_{1/3-(x+y)}\text{Cu}_x\text{Mg}_y)\text{O}_2$; ($x+y = 0.11$) and Ba substituted $\text{Li}(\text{Ni}_{1/3}\text{Mn}_{1/3}\text{Co}_{1/3-0.04}\text{Ba}_{0.04})\text{O}_2$, electrodes also showed promising applications, especially for high voltage lithium ion battery applications. The tested lithium ion battery cells assembled with anode and cathode electrodes developed have potential applications in lithium ion batteries with promising electrochemical performance.

The cycling behaviour of lithium ion coin cells assembled with developed Sri Lankan vein graphite anode and $\text{Li}(\text{Ni}_{1/3}\text{Co}_{1/3}\text{Mn}_{1/3})\text{O}_2$ cathode developed at C/5 rate between 3.0 - 4.2 V at room temperature is shown in Fig. 3. The cell assembled with vein graphite anode developed and synthesized cathode electrodes delivered an initial discharge

capacity of 100 mAhg^{-1} within a voltage window of 3.0 - 4.2 V, with a Columbic Efficiency of about 99%.

The study resulted in the successful development of material for the anode in Lithium Ion batteries, from purified Sri Lankan vein graphite and for the cathode, $\text{Li}(\text{Ni}_{1/3}\text{Mn}_{1/3}\text{Co}_{1/3-0.04}\text{Na}_{0.04})\text{O}_2$ from the Glycine Nitrate Combustion (GNC) process. The lithium ion cell assembled in this manner demonstrated promising electrochemical performance with good cycle stability

The study resulted in the successful development of material for the anode in lithium ion batteries, from purified Sri Lankan vein graphite and for the cathode $\text{Li}(\text{Ni}_{1/3}\text{Co}_{1/3}\text{Mn}_{1/3})\text{O}_2$ from the Glycine Nitrate Combustion (GNC) process. The rechargeable lithium-ion batteries assembled in this manner demonstrated promising electrochemical performance with good cycle stability.

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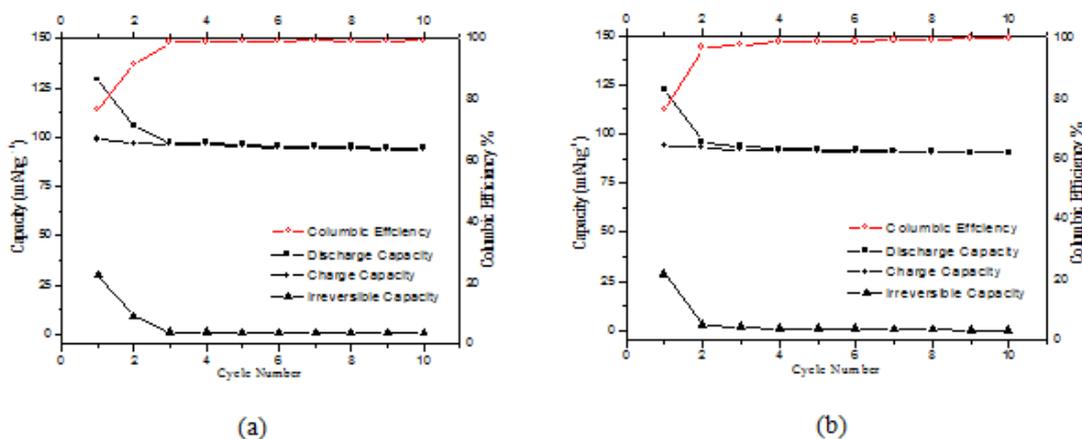


Figure 3. Electrochemical cycling performance of lithium ion coin cell fabricated with (a) purified Sri Lankan vein graphite anode (b) purified Sri Lankan vein graphite after mild oxidation with developed $\text{Li}(\text{Ni}_{1/3}\text{Mn}_{1/3}\text{Co}_{1/3})\text{O}_2$ as the cathode.

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Development and evaluation of fungal-bacterial biofilms as microbial ameliorators for potato cultivation



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In the last few decades application of chemical fertilizer (CF) in crop production has increased globally at an alarming rate. Since crop yields increase with the addition of fertilizer, the agricultural sector strongly depends on the application of mineral nutrients. Potato (*Solanum tuberosum* L.), a cash crop grown widely in Sri Lanka is considered as a heavy remover of soil nutrients as it takes up 1.5 times more nitrogen (N) and 4 to 5 times more phosphate than other vegetable crops. However, excessive use of CF and agrochemicals in conventional agriculture poses a major environmental threat globally, affecting the functioning of agroecosystems, resulting in a reduction in crop productivity due to the collapse of soil microbial diversity (Fig. 1).

As potato tubers grow entirely underground, there is a higher possibility for tubers to be contaminated by numerous toxic chemicals contained in chemical fertilizers. The application of beneficial microbial inoculants in the form of biofilmed bio-fertilizers (BFBFs) has been considered as an alternative to the excessive use of CF, nutrients and pesticides. The current study aimed to develop and evaluate BFBFs as an ameliorator/enhancer for crop productivity in potato, while improving soil quality. Initially, fungal-bacterial biofilms (FBBs) were developed, using beneficial microorganisms isolated from the rhizosphere of the potato crop, cultivated at the Regional Agriculture Research and Development Centre, Bandarawela.

Two biofilms were developed as biofertilizers; BFBF1 (with *Trichoderma harzianum* + *Bacillus pumilus* + *Bradyrhizobium japonicum* + *Bacillus subtilis*) and BFBF2 (with *Apergillus* sp. + *Serratia* sp. + *Bacillus* sp.) and were applied to potato fields in combination with chemical fertilizers (CF). Following the application of different BFBFs and CF combinations, growth and biochemical responses of potato plants, soil quality and rhizoremediation of nitrosamine and heavy metals in soil were evaluated. The study was carried out under greenhouse conditions, followed by field trials in three different climatic zones (Bandarawela, Bibile and Padukka) of the country (Fig. 2).

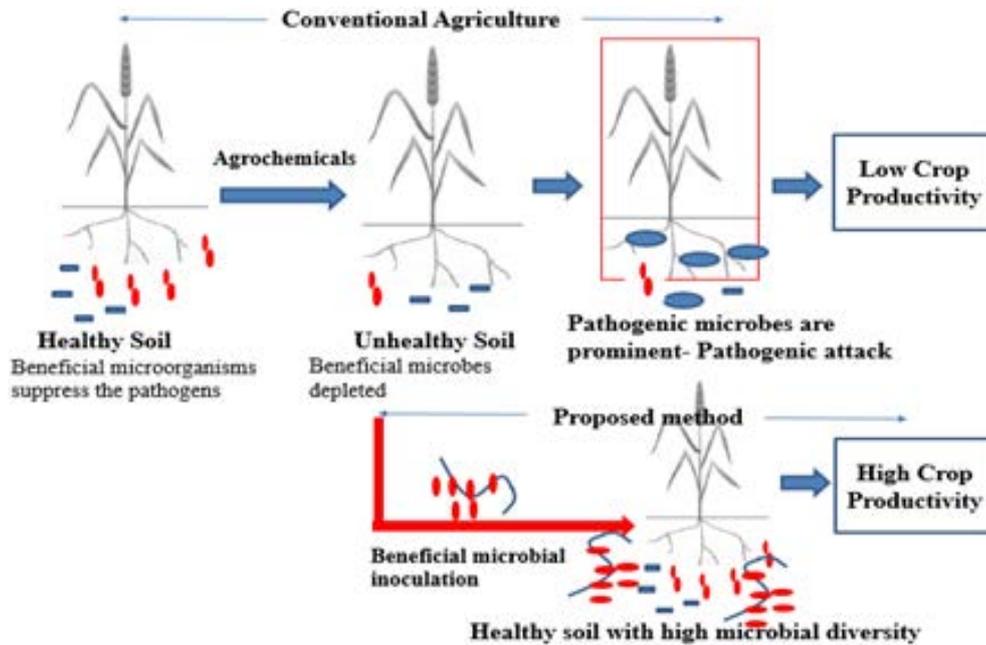


Figure 1. Schematic diagram illustrating the negative effects of chemical fertilizer application on soil microbial diversity and the proposed method to overcome the negative effects

Application of BFBF1 with 50% CF, to potato fields increased tuber weight in field sites in all three climatic zones, compared to the application of recommended CF (100%).

In Banadarawela, this treatment increased the potato yield up to 21 MT/ha, while the average potato yield

recorded with CF only, was 15-18 MT/ha. Although climatic conditions in Bibile are suitable for potato cultivation during the Maha season, the current study showed that the average tuber yield in the Bibile field sites was very low (only 2.67 MT ha⁻¹), while with the application of

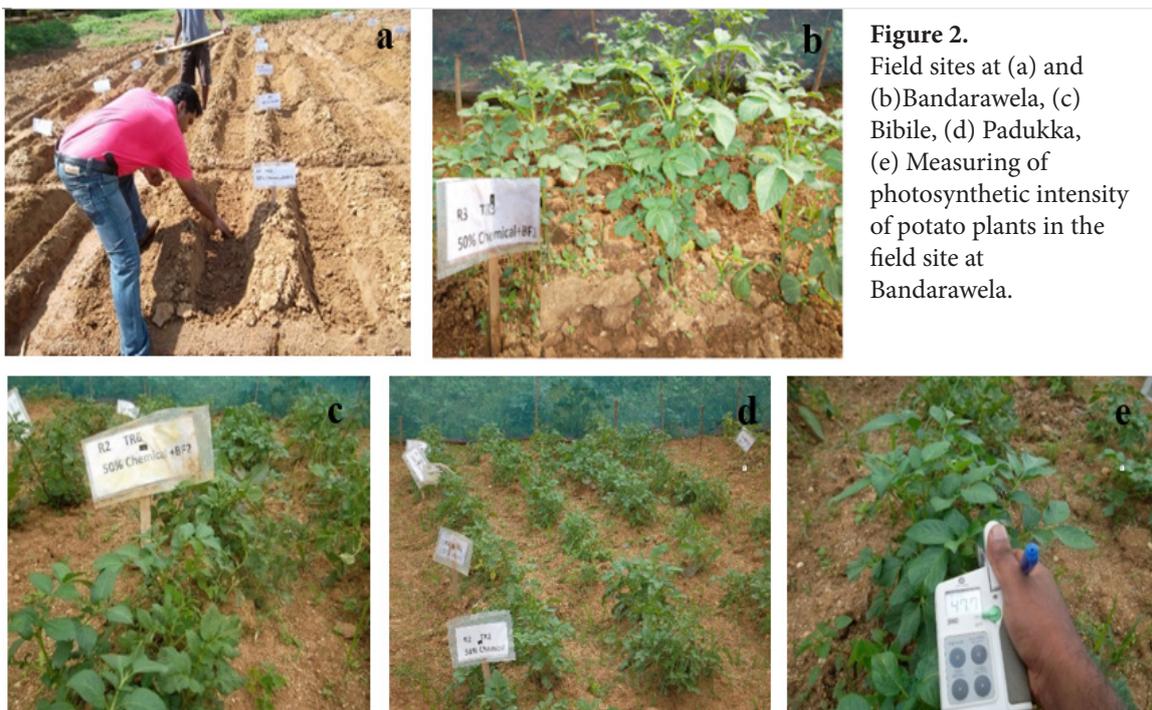


Figure 2. Field sites at (a) and (b) Bandarawela, (c) Bibile, (d) Padukka, (e) Measuring of photosynthetic intensity of potato plants in the field site at Bandarawela.

100% CF the yield was even lower (1.64 MT ha⁻¹). Moreover, yields from Padukka field sites were much lower (0.18 MT ha⁻¹), indicating that these areas are not suitable to establish large scale commercial potato cultivation.

The internal sugar and starch content of potato tubers were altered by BFBF1 + 50% CF, inducing tuberization. Enhanced tuberization was recorded following BFBF1 application in Bibile and Padukka field sites, where potato cultivation is not practiced. In these two areas, while there was a significant increase in tuberization with the application of BFBF1 + 50% CF, in comparison to the use of CF alone, tuber initiation did not lead to tuber growth or high yield.

Application of BFBF1 + 50% CF resulted in lower soil and plant tissue pH; higher soil organic carbon (SOC) content and microbial biomass carbon (MBC), and higher leaf chlorophyll and tissue calcium (Ca²⁺) content, compared to the application of 100% CF. The values of these parameters were similar in potato cultivations in all three field sites, irrespective of climatic conditions. Furthermore, soil Ca²⁺ content was significantly enhanced by the biofilm biofertilizer treatment, compared to 100% CF, in both Bandarawela and Bibile field sites. The biofertilizer treatment enhanced rhizoremediation of soil nitrosamines and heavy metals (Cd²⁺ and Pb²⁺). There was no increase in yield or in any of the parameters investigated with the application of BFBF2+ 50% CF, indicating that the composition of the biofilm biofertilizer is critical.

The observed increase in the yield is likely due to the exogenous favourable biochemical and physiological conditions created by BFBF1 induced internal biochemical mechanisms, leading to enhanced tuberization and tuber growth, while improving rhizo-remediation. Further, the climatic requirements for potato tuberization have been compensated by the biofilm microbial actions. The findings confirm that the use of chemical fertilizer can be reduced by 50% with the use of the biofilm biofertilizer

(BFBF1) developed, resulting in environmental and economic gains, while improving soil health.

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Effectiveness of rizobial strains on the yield of three major legume crops grown in Sri Lanka



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The use of urea as a nitrogen fertilizer has increased dramatically during the past 45 years and it is now the most important fertilizer in agriculture, world over. However, indiscriminate use of urea has led to many environmental and health problems. In Sri Lanka, the government is spending much foreign exchange on importing urea and is distributed to the farmers at a subsidiary rate. Therefore, replacing urea with suitable alternatives such as the use of rhizobial inoculants, is a timely option.

The current study was undertaken to develop effective rhizobial inoculants for three major legume crops grown in Sri Lanka, namely, black gram (*Vigna mungo*), green gram (*Vigna radiata*) and soybean (*Glycine max*). To achieve this goal, the following procedure were carried out: (i) establishment of a reference germplasm collection of native rhizobia, in order to characterize and identify the isolated rhizobia, (ii) evaluate the stress tolerance ability of isolates, (iii) identify the isolates at molecular level and authenticate the rhizobial isolates, (iv) screen isolates for symbiotic effectiveness in sand culture, (v) determine the success of cross inoculating crop legumes using rhizobial strains isolated from wild legumes and (vi) evaluate the efficiency of single strain and multi strain rhizobial inoculants in crop production and yield of black gram, green gram and soybean.

Rhizobial isolates were obtained from root nodules of several cultivars of legumes; edible legumes, non-edible wild legumes and wild relatives of *Vigna* spp. collected from 34 agro-ecological zones, representing major areas of food legume cultivation in Sri Lanka (Fig. 1). Reference germplasm collection of 777 stock cultures of native rhizobia were obtained (Fig. 2). Several characteristics of the collected 143 rhizobial isolates were determined; intrinsic resistance to antibiotics, carbon source utilization and tolerance to pH, salinity, temperature and drought, followed by molecular characterization (Fig.3). Testing for authenticity and screening for infectivity and effectiveness of the isolates were carried out in sand cultures as pot experiments (Fig. 4).



Figure 1. Collection of root nodules of legumes in the field

A total of 10 strains, isolated from non-edible wild legumes (6 strains) and wild relatives of *Vigna* spp. (4 strains) identified to be infective and/or effective and stress tolerant, were selected for cross inoculation and the most suitable strain/s for each of the three legume crops were selected.

Molecular characterization revealed that the 10 selected strains were different from each other. They were field tested as single strain and multi strain inoculants in a field at the Department of Botany, Faculty of Science, University of Peradeniya and in different locations in the Dry Zone of Sri Lanka,

under farmer practices (black gram and green gram at Mahalluppallama and Bulagala and Soybean at Mahalluppallama and Galnawa)

Addition of coir based rhizobial inoculants (Fig. 5) resulted in increases in the growth performances of all treatments when compared to the N-control and in certain instances gave significantly higher ($p \leq 0.05$) increases than the N fertilizer treatment. Table 1 gives the yield comparison with N+ application in field trials at the Department of Botany, University of Peradeniya, while Table 2 gives the yield comparison with N+ application in field trials in farmer fields.



Figure 2. Pure culture (left), Broth cultures (center), Stock cultures (right) of rhizobia

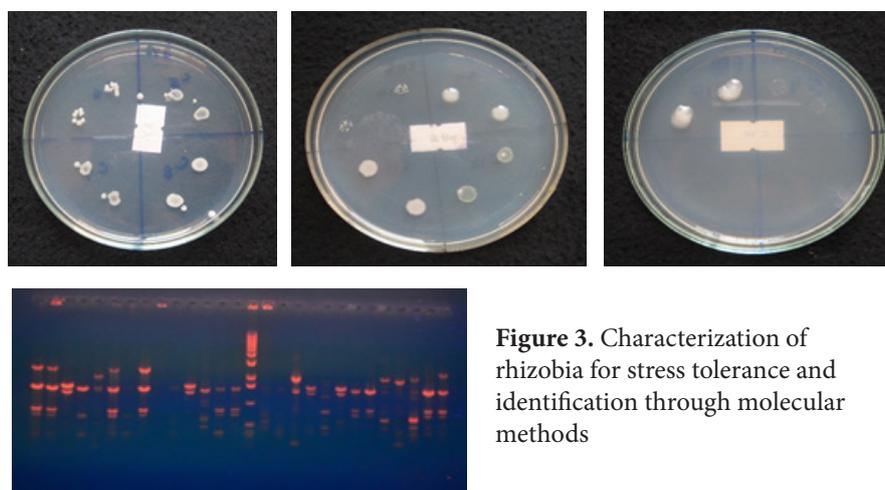


Figure 3. Characterization of rhizobia for stress tolerance and identification through molecular methods



Figure 4. Plant house experiment / Field trials



Figure 5. Coir based rhizobial inoculum packets

Overall, inoculation with single strains 2, 1 and 3 individually, gave significantly higher yields that ranged from 4% -16%, 3% - 4% and 7% - 14% respectively, for each of the crops (black gram, green gram and soybean) over N fertilizer treatment. In black gram, 2 single strain inoculants and 3 multi strain inoculants gave significant yield increases ranging from 3% to 39%, compared to N fertilizer application. In green gram, the yield increases ranged from 5% to 14%, with a single strain inoculant and multi strain inoculants resulting in significantly higher yields. In soybean, yield increases were significantly higher and ranged from 4% to 13%, with 2 single strain inoculants and 2 multi strain inoculants compared to N fertilizer

Table 1. Yield comparison with N+ application in field trials at the Department of Botany, University of Peradeniya.

Black gram		Green gram		Soybean	
Strain	Comparison %	Strain	Comparison %	Strain	Comparison %
M3	0.64	M6	4.05	C10	21.73
M6	-2.34	VD1	1.13	M5	12.14
VD1	4.25	VW1	1.8	VD1	26.18
VW1	-1.06	VW2	3.38	VW2	11.74

Table 2. Yield comparison with N+ application in field trials in Farmer fields

Black gram		Green gram		Soybean	
Strain	Comparison %	Strain	Comparison %	Strain	Comparison %
M3	0.64	M6	4.05	C10	21.73
M6	-2.34	VD1	1.13	M5	12.14
VD1	4.25	VW1	1.8	VD1	26.18
VW1	-1.06	VW2	3.38	VW2	11.74
VD1	16.67	VW1	3.32	M6	-1.67
VW1	4.17	VW2	3.52	VD1	14.91
VW2	-4.17			VW2	-0.21

Table 3. Yield comparison of single strain and multi strain rhizobial inoculants applied to the three legume crops

Crop legume	Yield increases over N+ control	
	Single strain rhizobial inoculants %	Multi strain rhizobial inoculants %
Black gram	3 - 34	8 - 39
Green gram	5- 14	5 - 12
Soybean	4	12 - 13

treatment. In both black gram and soybean, higher yields were obtained with multi strain inoculants compared to single strain inoculants, while in green gram, increases in yield were obtained with both single and multi strain inoculants (Table 3).

The study highlights the important role played by rhizobial inoculants, obtained from field collected, local rhizobial isolates. Their ability to effectively replace urea fertilizer application in black gram, green gram and soybean has a high potential in the cultivation of these legumes in Sri Lanka.

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Improving bioavailability of bioactive components for drug and cosmetic based applications



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Nanotechnology is an innovative approach that has potential applications in many spheres. To improve the bioavailability of bioactive components in drug and cosmetic based products, use of novel carrier systems based on nanotechnology is yet another innovative approach. New carrier systems developed in this manner have the ability to overcome many drawbacks associated with conventional methods such as toxicity, high dose, and repetitive dosage. The first part of the study is an attempt to improve the bioavailability of an anticancer drug through the use of biodegradable, biocompatible polysaccharides, such as chitosan and alginate. For the encapsulation of the anticancer drug, doxorubicin, two polymeric nano systems were synthesized, namely, chitosan nanoparticles and chitosan-alginate nanoparticles. In the second part of the study, the main goal was to develop a liposomal formulation for the antioxidant caffeic acid thereby, improve its efficiency through increased skin permeation. In the last part of the study, attempts were made to develop a safe carrier system for iron, using a biocompatible, biodegradable polymer.

Thus the objectives of the study were to (i) Develop a novel carrier system for the anti-cancer drug (doxorubicin), (ii) To prepare and characterize doxorubicin loaded chitosan and chitosan-alginate nanoparticles, (iii) To check slow release behaviour of the drug from nanoparticles and (iv) To determine the cytotoxicity of both drug loaded systems against MCF-7 (breast cancer) cell line.

For the improved delivery of caffeic acid (antioxidant) through skin by liposomal encapsulation, the following procedure was followed: (i) Preparation of caffeic acid loaded liposomes with cholesterol and egg lecithin and to characterize the loading and the formation of liposomes, (ii) Determination of the release behaviour of caffeic acid from liposomes and the improved skin permeation using a pig ear skin and (iii) Comparison of the antioxidant activity of caffeic acid after encapsulation and skin permeation. For the development of a novel oral carrier system for iron, ferrous-loaded alginate nanoparticles were prepared and characterized, and the slow release behaviour of

ferrous in different pH buffer solutions (pH 7.4 in stomach and pH 6 in duodenum) were determined.

A novel carrier system for the anti-cancer drug (doxorubicin)

Chitosan nanoparticles were synthesized according to a previously described, two stepped method; oil in water emulsion followed by cross linking. Thereafter, DOX loaded chitosan-alginate nanoparticles were prepared by a novel method. The drug loaded was determined by fluorimetry. The two types of nanoparticles were characterized for particle size, morphology and successful drug loading. The slow release behaviour of the drug, loaded with the two types of nanoparticles was monitored in pH 7.4 buffer solution. The cytotoxicity of drug loaded nanoparticles were compared with the free drug on MCF-7 breast cancer cells.

Delivery of caffeic acid (antioxidant) through skin by liposomal encapsulation

Caffeic acid encapsulated liposomes were prepared using the reverse phase evaporation method. The amount of caffeic acid loaded was determined by UV-Visible spectrophotometry. Liposomes were characterized for particle size and morphology. Slow release of caffeic acid was monitored in pH 7.4 buffer solution. Skin permeation ability of liposomal caffeic acid was determined using pig ear skin mounted Franz cell. The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay was performed according to a previously described procedure to compare the antioxidant activity of encapsulated caffeic acid with standard caffeic acid.

A novel oral carrier system for iron

Ferrous-loaded alginate nanoparticles were prepared using ionic gelation method. Amount of

iron loaded was quantified by spectrophotometry. Iron-loaded nanoparticles were characterized for size, morphology and successful loading. Slow release behaviour of iron was monitored in buffer solutions of different pH (stomach pH and duodenum pH).

Developed nanoparticles

The size of the two types of nanoparticles developed was < 20 nm and were spherical in shape (Fig. 1). Due to the encapsulation with nanoparticles, the release of doxorubicin was significantly retarded (Fig. 2).

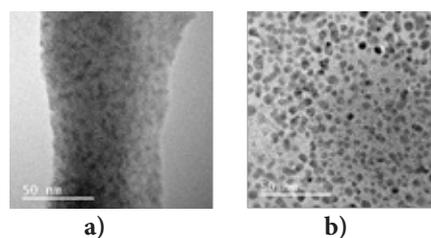


Figure 1. Transmission electron microscopy images of (a) chitosan (b) chitosan-alginate nanoparticles

Doxorubicin encapsulated in nanoparticles behaved differently from the un-encapsulated/ free-state form, both having similar concentrations of the drug. The encapsulated form released the drug slowly and gradually increasing the concentration of the drug in the medium (Fig. 3). Thus, confirming that encapsulation of the drug through either system has improved the efficacy of DOX over free DOX.

Improved delivery of caffeic acid (antioxidant) through skin by liposomal encapsulation

Prepared caffeic acid loaded liposomes showed

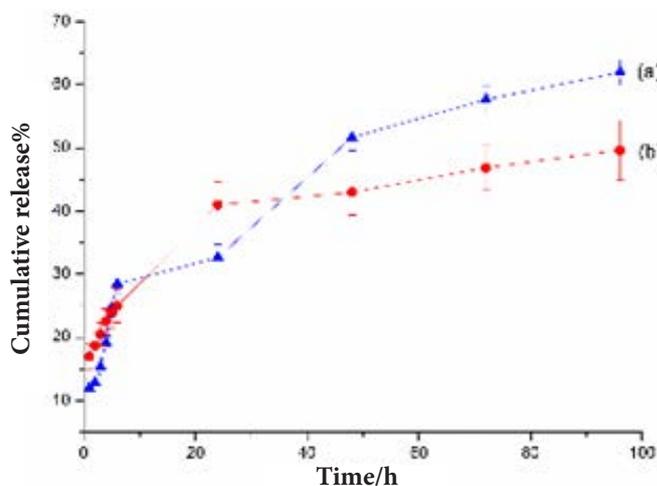


Figure 2. Controlled cumulative release of doxorubicin from (a), chitosan-alginate and (b), chitosan nanoparticles at preselected time intervals in pH 7.4 buffer.

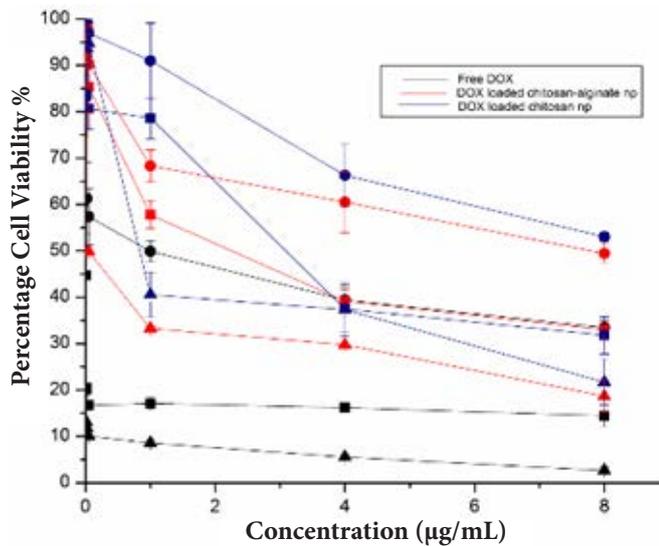


Figure 3. Cytotoxicity of free drug, drug loaded chitosan-alginate nanoparticles and chitosan nanoparticles against MCF-7 cells after 24, 48 and 72 h treatments.

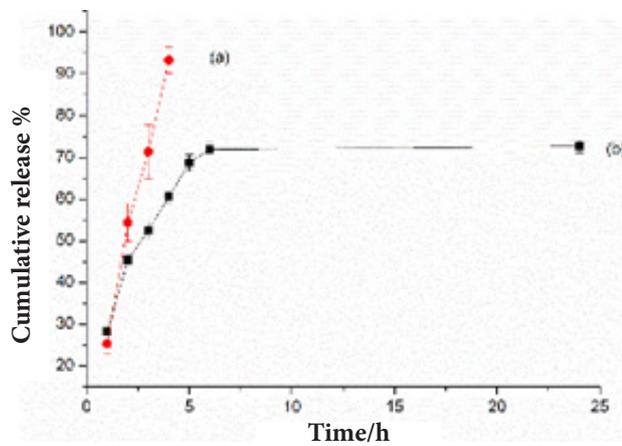


Figure 4. Diffusion profile of caffeic acid through dialysis membrane (a) free caffeic acid and (b) liposomal caffeic acid

spherical shaped 3D globular vesicles well dispersed without aggregation (Fig. 5). Liposomal caffeic acid showed a slow, steady release over 6-7 h while free caffeic acid diffused rapidly and almost 50% had diffused after 2 h (Fig. 4). A considerable amount of liposomal loaded caffeic acid ($41.8 \pm 1.58\%$) had permeated slowly through the epidermis during 7 h while only $2.0 \pm 0.02\%$ of caffeic acid had deposited in the skin. Free caffeic acid penetration was $5.3 \pm 1.91\%$ which is significantly lower than liposomal caffeic acid (Fig. 6).

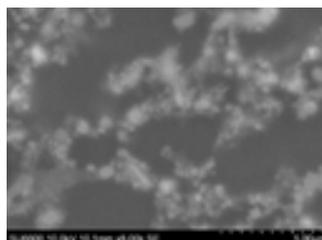


Figure 5. SEM images of caffeic acid loaded liposomes

Development of a novel oral carrier system for iron

The spherical shape and smooth surface of the nanoparticles with no observed aggregation was clearly visible (Fig. 7).

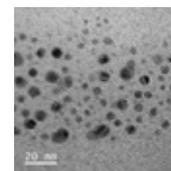


Figure 6. TEM image of ferrous loaded alginate nanoparticles

The total amount of ferrous released from alginate nanoparticles at pH 7.4 buffer solution was around 65% after 96 h, while it was around 70% at pH 6 and at pH 2, the released amount was $< 20\%$. Overall, findings of the study infer that (i) Chitosan-alginate nanoparticles have the potential to be developed as a slow release carrier system for the drug doxorubicin,

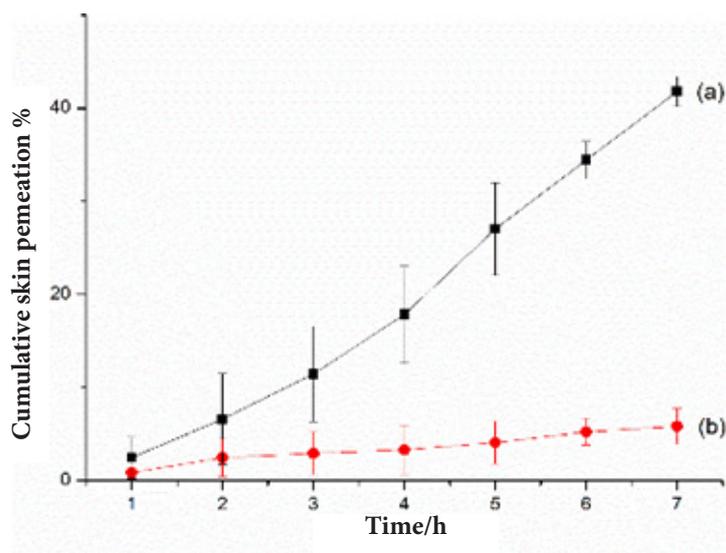


Figure 7. Penetration profile of caffeic acid through pig ear epidermis (a) liposomal caffeic acid and (b) free caffeic acid

(ii) Liposomal caffeic acid formulation has the potential to be developed as a skin cream formulation that successfully slows down the complex process of photooxidative skin damage and (iii) Ferrous-loaded alginate nanoparticles provide an attractive delivery system for conventional oral iron therapy.

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Investigations on Hermitian and non-Hermitian isospectral counterparts



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The Wentzel–Kramers–Brillouin (WKB) approximation method is found to be one of the useful methods for obtaining approximate energy eigenvalues and eigenfunctions of the 1-D Schrödinger equation. This method was extended to obtain higher-order eigenenergies by other researchers. Generally, for polynomial potentials, the direct application of the WKB method to obtain higher-order terms in the expansion is found to be very complicated (if not impossible) due to the fact that integrals in the expansion coefficients cannot be evaluated analytically. However, recently developed Asymptotic Energy Expansion method (AEE) provides the necessary tools to overcome this difficulty. AEE is useful in obtaining analytic expressions for quantum eigenenergies for 1-D potentials.

Conventionally, it is assumed that a quantum mechanical Hamiltonian must be Hermitian in order to faithfully describe a physical system. A Hermitian Hamiltonian is self-adjoint with respect to the standard, Hermitian inner product of quantum mechanics. This is a sufficient condition for the reality of the energy spectrum. Recently, this condition has been challenged as being too restrictive. Previous researchers have showed, using numerical techniques, that the spectrum of

$$H = p^2 + x^2(ix)^\varepsilon$$

is real and positive for $\varepsilon \geq 0$. This result was later given formal proof using techniques from the theory of integrable systems. The class of Hamiltonians is clearly not Hermitian but possesses an antilinear PT -symmetry invariance under simultaneous parity (P) and time reversal (T). On this basis, it was proposed that Hermiticity is replaced with the weaker requirement of PT -symmetric. The search for finding a condition which is both necessary and sufficient for the reality of the spectrum has led to a notion of a pseudo-Hermitian operator. According to a definition proposed, H is called pseudo-Hermitian, if there exists a linear, Hermitian and invertible operator $\eta: \mathbb{H} \rightarrow \mathbb{H}$ satisfying

$$H^\dagger = \eta H \eta^{-1}$$

where \dagger denotes the adjoint of the corresponding operator and η is called the metric operator. Such a metric operator is clearly positive-definite and defines a positive-definite inner product $\langle \cdot, \cdot \rangle := \langle \cdot | \eta \cdot \rangle$, where $\langle \cdot | \cdot \rangle$ is the defining inner product of the Hilbert space in which H and η act. The metric operator can be used to construct a Hermitian with respect to the “usual” inner product operator equivalent to the given pseudo-Hermitian Hamiltonian

$$h = \rho H \rho^{-1}$$

where $\rho = \sqrt{\eta}$. The non-Hermitian Hamiltonians having this property are called quasi-Hermitian. In general, for a given non-Hermitian Hamiltonian H , the similarity transformation, is not unique when the only requirement for h is its Hermiticity.

Having analyzed the role that PT -symmetric Hamiltonians could play within a quantum framework, it is interesting to examine those Hamiltonians in the context of classical mechanics. In recent years, there has been a growing interest in the study of non-Hermitian complex classical mechanics, and possible applications to new physical systems. The major reason for the interest is that, through the use of PT -symmetry in the place of Hermiticity, the possible number of Hamiltonians that could be investigated is greatly increased.

It has been proven that a quantum system possessing an exact (unbroken) PT -symmetry is equivalent to a quantum system having a Hermitian Hamiltonian. This was achieved by constructing a unitary operator relating an arbitrary non-Hermitian Hamiltonian with exact PT -symmetry to a Hermitian Hamiltonian. Nevertheless, only in a few instances, it is possible to find Hermitian Hamiltonians which possess the same eigenspectra as PT -symmetric non-Hermitian Hamiltonians. The main objective of this study was to determine the isospectral Hermitian counterparts for non-Hermitian Hamiltonians using several methods. In addition, the study has the following two specific objectives; firstly, to construct the Hermitian and non-Hermitian isospectral counterparts in terms of Euclidean E_2 -Lie algebraic generators and secondly to find the classical counterparts of non-Hermitian PT -symmetric quantum theory.

Several years ago, by using operator techniques and path integral methods, researchers proved that the eigenspectra of non-Hermitian PT -symmetric

Hamiltonian with a “wrong-sign” quartic potential and the Hermitian Hamiltonian with a positive quartic potential together with a linear term are the same. In this study, the AEE method and the Algebraic techniques were used to show that the Hermitian and non-Hermitian isospectral counterparts.

The recently developed semiclassical AEE method has been used to show that the complex non-Hermitian PT -symmetric Hamiltonian $p^2 - gx^4 + 4i\hbar\sqrt{gx}$ and the conventional Hermitian Hamiltonian $p^2 - gx^4 + 4i\hbar\sqrt{gx}$ have the same eigenspectra. Similarly, using AEE, it has been shown that the non-Hermitian Hamiltonians $H = p^2 - gx^4 + a/x^2$ and the conventional Hermitian Hamiltonians $h = p^2 + 4gx^4 + bx$ ($a, b \in \mathfrak{R}$) are isospectral if $a = (b^2 - 4g\hbar^2)/16g$ and $a \geq -\hbar^2/4$. When $a = (b^2 - 4g\hbar^2)/16g$ and $a < -\hbar^2/4$ although h and H are still isospectral, b is complex and h is no longer the Hermitian counterpart of H . Further, in order to find the isospectral Hermitian counterparts, the AEE method is successfully extended to derive an almost explicit analytic formula for semiclassical energy expansion of general odd degree polynomial potentials. In general, AEE method produces accurate eigenvalues for the higher eigenstates and parameters of the non-leading power terms of the potential are small. This study shows that by applying Padé approximation, the accuracy of AEE for the ground state and low eigenstates can be significantly improved and eigenenergies for large parameters can be accurately determined.

Several classes of non-Hermitian and Hermitian isospectral counterparts, which can be expressed in terms of bilinear combinations of Euclidean E_2 -Lie algebraic generators were studied. The classes are distinguished by different versions of antilinear (PT) symmetries exhibiting various types of qualitative behaviour. On the basis of explicitly computed non-perturbative Dyson maps, metric operators were conducted and found the isospectral Hermitian counterparts. Further, regions in the parameter space for which the corresponding spectra are entirely real and also domains where the PT -symmetry is spontaneously broken, were identified.

It is generally true that even for real energies, classical trajectories of non PT -symmetric Hamiltonians with complex parameters are mostly non-periodic and open. This study showed that for given real energy, the classical trajectories of complex quartic Hamiltonians,

$H = p^2 + ax^4 + bx^k$ (where a is real, b is complex and $k = 1$ or 2) are closed and periodic only for a discrete set of parameter curves in the complex b -plane. Furthermore, it was found that given complex parameter b , the classical trajectories are periodic for a discrete set of real energies. Moreover, it is shown that, for real and positive energies (continuous), the classical trajectories of complex Hamiltonian $H = p^2 + \mu x^4, \mu = \mu_r e^{i\theta}$ are periodic when $\theta = a \tan^{-1}[(n/(2m+n))]$ for $\forall n$ and $m \in \mathbb{Z}$.

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Incorporation of bioactive compounds in emulsions for application in cosmetic industry



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Drug delivery vehicles play pivotal roles in the pharmaceutical industry. Likewise, bioactive agent delivery vehicles play crucial roles in other multi-million industries, such as cosmeceutical nutraceutical and food. The present study centered on liposomes - a delivery vehicle used in the delivery of drugs, bioactive agents and numerous other materials that have a multitude of significant applications.

Liposomes are spherical structures made up of one or more lipid bilayers with an aqueous interior. These are closed vesicles enclosing an internal aqueous space. The internal compartment is separated from the external medium by a bilayer membrane. The lipid bilayers are composed of discrete lipid molecules. The lipid component of liposomes, usually, consists of phospholipids and lipid-soluble compounds while the aqueous component consists of an aqueous solution with water-soluble compounds. The main advantage of liposomes is that they consist of both a lipid component as well as an aqueous component, and hence, both lipid soluble components and water soluble components can be loaded into these vesicles. A typical unilamellar liposome is shown in Fig. 1.

As the material to be loaded to liposomes, compounds having potential applications in pharmaceutical, cosmeceutic, nutraceutical and food industries can be selected. In the present study, the model bioactives selected were vitamin E, ferulic acid and curcumin which are potent antioxidants. In addition, a highly antioxidant plant extract from *Schumacheria castaneifolia* was also used.

The objective of liposomal encapsulation of bioactive agents was to enhance or optimize the properties of the bioactives, significant to areas of application as stated here. The properties considered in this study were the particle size, zeta-potential, encapsulation efficiency, loading capacity, in vitro release properties and skin permeation properties. Although most of these attributes are important to a number of fields, skin permeation in particular is vital in cosmetic, cosmeceutic and in certain pharmaceutical industries.

In order to achieve this goal, initially, liposomes were prepared using three different methods; reverse-phase evaporation method, thin-film hydration method and/or proliposome method. Thereafter, variations in the properties of liposomes were evaluated with respect to changes in the lipid composition of liposomes. In vitro release studies were carried out using the dialysis bag method while ex vivo skin permeation experiments were carried out with pig ear skin, as a substitute for human skin, using a Franz-diffusion cell.

A key finding of the liposomal studies was that the properties of the liposomal bioactives depended heavily on the lipid composition of liposomes. The presence of cholesterol in the lipid bilayer may have affected lipid packing and encapsulation of methanol extract of stem-bark of *Schumacheria castaneifolia*. Similarly, interactions between stearylamine in the lipid bilayer and ferulic acid may be the reason for the high encapsulation efficiency of ferulic acid loaded positively charged liposomes. Encapsulation efficiencies of curcumin encapsulated liposomes also appeared to be dependent on the interactions of curcumin and lipids. Attributes, such as loading capacity, size, zeta-potential, polydispersity index, release, and skin permeation properties were lipid composition dependent.

Much emphasis is placed upon the drug/bioactive release properties of delivery vehicles in numerous fields due to a number of reasons. For instance, sustained release is desired in pharmaceuticals and cosmeceutics due to reduced or no toxic effects, and reduced dosing frequency. In the food industry, sustained release properties are

important in facilitating consistency and protecting susceptible loaded material. This study revealed that the release kinetics of encapsulated species may be modulated by changing the lipid composition. For instance, ferulic acid and curcumin loaded positively charged liposomes exhibited slower release.

The outermost layer of the skin, stratum corneum, acts as the barrier between the external environment and the human body. Consequently, facilitating skin deposition and skin penetration of bioactive agents is very challenging. This study evaluated emulsions and liposomes as a means of increasing skin permeation of bioactives. Liposomal vitamin E incorporated in emulsions showed superior skin penetration properties compared to liposomal vitamin E and vitamin E incorporated emulsion. Moreover, skin permeation of ferulic acid and curcumin showed dependence on lipid composition and charge of liposomes. In fact, negatively charged liposomes showed higher skin permeation properties.

The second part of the study involved binary systems, such as cocrystals, solid solutions and eutectics that have interesting applications, especially, in pharmaceuticals, nutraceuticals and cosmeceutics. The aim was to form cocrystals to enhance mainly the solubility of curcumin, that would lead to increased bioavailability. The co-former used in this study was ferulic acid which is a structural analogue of curcumin. The chemical structures of curcumin and ferulic acid are shown below.

Cocrystal formation using curcumin and ferulic acid was attempted using solvent evaporation, liquid-

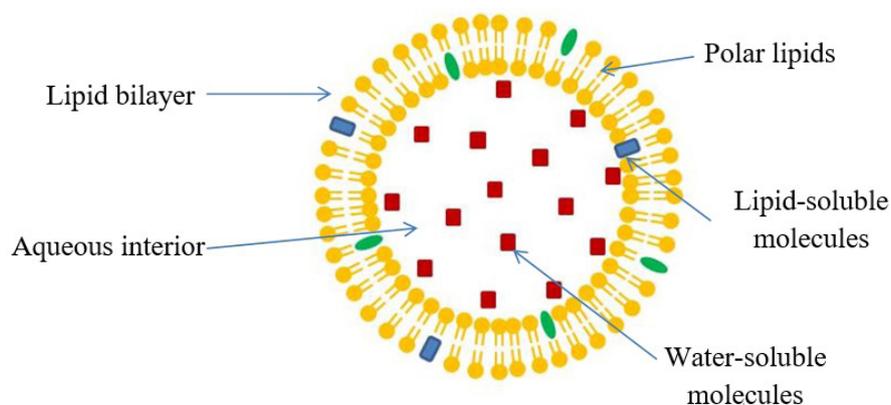
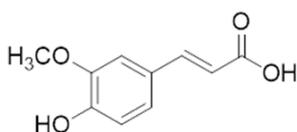
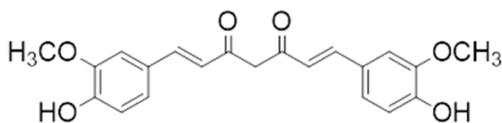


Figure 1. A cross section of a unilamellar liposome



Structure of ferulic acid



Structure of curcumin

assisted grinding and neat-grinding. The products were characterized using SEM, PXRD, FT-IR, TGA and DSC. Solvent evaporation yielded either a cocrystal or a solid solution while grinding methods yielded eutectics. Dissolution studies revealed that solid solution or eutectic formation increases the dissolution rate of curcumin, and that the three methods used are equally effective. Furthermore, using a mixture of curcumin and ferulic acid confers photostability to curcumin. Also, a 1:1 (mol/mol) mixture of curcumin and ferulic acid exhibited enhanced antioxidant potential even after exposure to UV radiation. Basically, curcumin and ferulic acid, when used as a binary system, improve significant properties, such as the dissolution rate and antioxidant potential.

In summary, this study portrays the ability of liposomes and binary systems, such as cocrystals, solid solutions and/or eutectics to enhance pharmaceutically, nutraceutically and cosmeceutically relevant physical properties of antioxidants.

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Characterization of biochemical expressions in fungal-bacterial-plant interactions with special reference to microbial biofilm formation



I. Darshani Singhalage graduated with a BSc Special Degree in Botany from the University of Peradeniya, in 2005. Darshani worked at the Royal Botanic Gardens, Peradeniya for a short period as a research assistant. She joined the Department of Science and Technology, Uva Wellasa University of Sri Lanka, in 2007 as a Lecturer and rose to the rank of Senior Lecturer. She embarked on her postgraduate studies focusing on fungal-bacterial-plant interactions and obtained her PhD Degree from the PGIS in 2015. Email: idsinghalage@yahoo.com

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Organisms are able to communicate with each other through mutualism, parasitism or commensalism. Mutualism and commensalism are beneficial and have many applications in diverse fields such as agriculture, forestry, environment protection, biotechnology and medicine. Diffusible signals and physical interactions among organisms can induce silent biosynthetic genes by making them metabolically heterogeneous. Mixed biofilms containing both fungi and bacteria can be considered as the most intimate level of microbial association termed as Fungal-Bacterial Biofilms (FBBs). Usage of FBBs as Biofilmed Biofertilizers (BFBBs) has been introduced and recently they have many benefits. However, tripartite interaction among bacteria-fungi-plant root has not been extensively studied. Therefore, in the present study this interaction and its quorum sensing-based communication were investigated. In addition, induced metabolic activity due to this interaction and the usage of its metabolomics in strawberry growth promotion has been investigated.

The study was conducted with the objective of investigating the structural and functional attributes, including biochemical expression and quorum sensing in biotic interactions among fungi, bacteria and plant. Cultivated and wild strawberry rhizosphere associated fungi and bacteria were isolated and screened for strawberry growth promotion. Fungal-bacterial biofilms (FBBs) were developed using these microbes. The potential use of the developed FBBs as biofilmed biofertilizers (BFBBs) was evaluated with different dosages of chemical fertilizers recommended for strawberry, using glasshouse and field experiments. Promising biofilms were identified and their biochemical expressions and structural attributes were studied using Fourier Transform Infra-red (FTIR) spectroscopy. FTIR was also used to investigate the functional properties between strawberry, rice and tomato root-biofilm interactions for assessing the use of BFBBs universally. Quorum sensing of bacterial and fungal-bacterial biofilms, and also biofilm-root interactions was



Figure 1. Interactions between *Enterobacter* - *Aspergillus* biofilm, seen under light microscopy. A fungal filament attached by bacteria forming a biofilm.

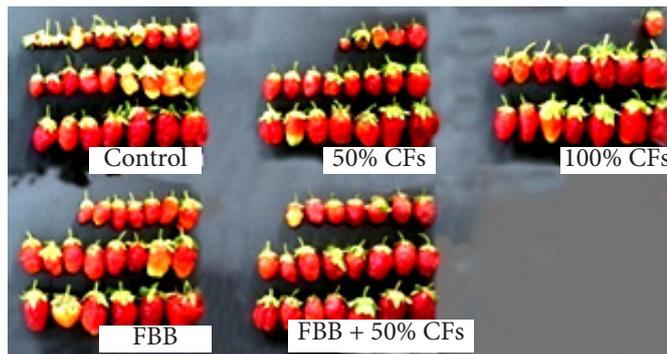


Figure 2. Differences in size and number of strawberry fruits harvested under the four treatments (50% CFs, 100% Cfs, FBB alone and FBB + 50% CF) and the control.

investigated by using bio-sensing experiments.

A simple FBB (Fig. 1) developed from strawberry growth promoting *Enterobacter* spp. and *Aspergillus* spp. together with 50% recommended chemical fertilizers significantly improved the strawberry yield in glasshouse experiments. The same treatment improved the quality and quantity of fruits compared to 100% chemical fertilizer application in the field trials (Fig. 2). In terms of productivity, strawberry with FBB was 152% more profitable compared to 100% chemical fertilizer

treatment in the glasshouse experiment, while it was 31% more profitable in the field experiments. When the structural properties of biofilms were considered, structural polysaccharides, amides and fatty acids drastically increased during the first three days of biofilm formation before reaching a static phase eventually. Bacterial biofilm and FBB showed characteristic amide I and II bands, whereas fungal biofilm displayed only amide I band (Fig. 3). In the Cluster analysis, the functional properties of FBB were separately clustered indicating different functional

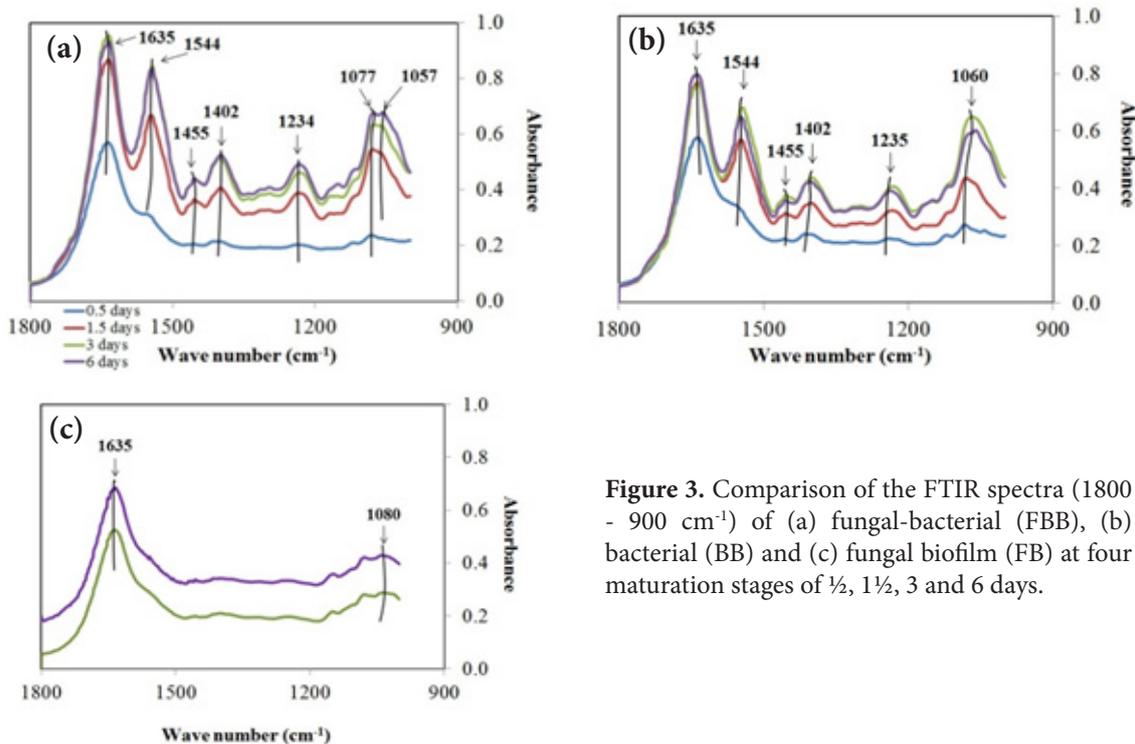


Figure 3. Comparison of the FTIR spectra (1800 - 900 cm⁻¹) of (a) fungal-bacterial (FBB), (b) bacterial (BB) and (c) fungal biofilm (FB) at four maturation stages of ½, 1½, 3 and 6 days.

attributes of FBB compared to those of fungal or bacterial biofilms (Fig. 4). FBB developed from strawberry rhizospheric microbes improved vegetative growth (Fig. 5) of tomato and rice plants compared to the bacterial or fungal biofilms, indicating its universal applicability. Bio-sensing experiments together with FTIR identified two homoserine lactones (AHL) as quorum sensing molecules involved in the formation

of bacterial and fungal-bacterial biofilms (Fig. 6).

The overall findings of the study infer that the intimate physical interactions among bacteria, fungi and plants have released a diverse array of functional metabolites that are involved in enhancing plant growth.

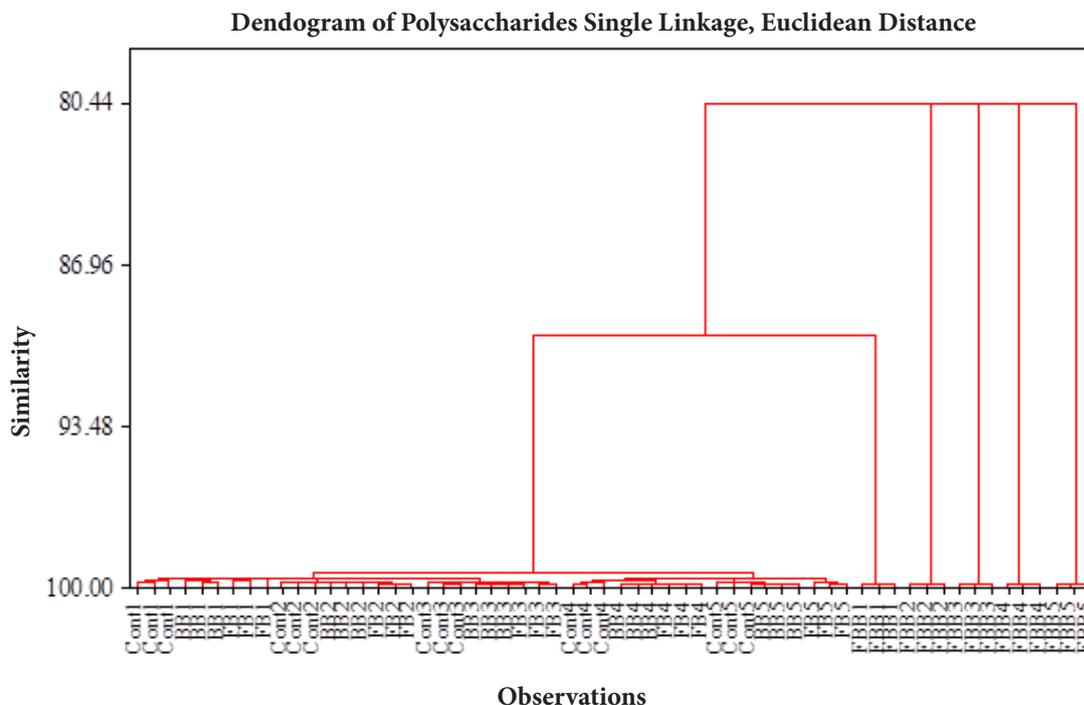


Figure 4. Dendrogram showing hierarchical cluster analysis of FTIR spectral functional groups of polysaccharide exudates (900 - 1200 cm^{-1}) of bacterial (BB), fungal (FB) and fungal - bacterial (FBB) biofilms and control at five ($\frac{1}{4}$, $\frac{1}{2}$, $1\frac{1}{2}$, 3 and 6 days) harvests. Number followed by each treatment and control represent the harvesting times (1 = $\frac{1}{4}$, 2 = $\frac{1}{2}$, 3 = $1\frac{1}{2}$, 4 = 3 and 5 = 6 days). Similar clustering patterns were observed for functional amides and functional lipids.

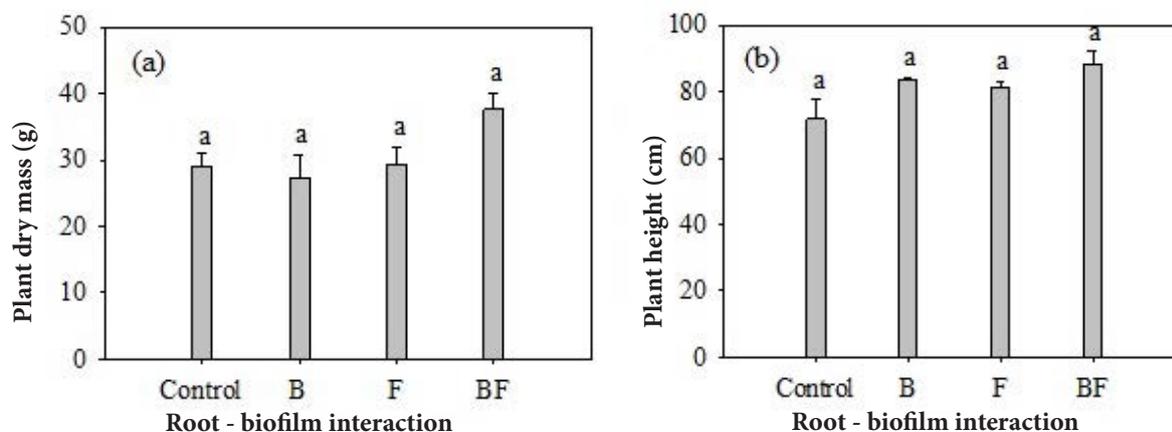


Figure 5. Dry mass (a) and height (b) of tomato seedlings at harvest. BB – bacterial biofilm, FB - fungal biofilm, FBB-fungal–bacterial biofilm. Columns headed by the same letter are not significantly different at 5% probability level.



Figure 6. Field experiment at SeethaEliya Agricultural Research Station

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Evaluation of antimicrobial activity of Parankipaddai Kudineer (decoction) used in skin diseases



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Sri Lanka

Parankipaddai kudineer (PPK) is a decoction used in Siddha medicine. It is recorded in the 12 volume classic “Pararajasekaram siraroganithanam”, written in poetic form. It is mentioned as a treatment for skin diseases in this classic work. It is prepared from parts of 12 medicinal plants, namely, *Smilax chinensis*, *Indigofera aspalathoides*, *Encicostemma littorale*, *Cassia angustifolia*, *Azima tetraacantha*, *Embelia ribes*, *Zingiber officinale*, *Piper nigrum*, *Piper longum*, *Myristica fragrans* (seed and leaves), *Syzygium aromaticum* and *Hyoscyamus niger*. Although PPK is used in the treatment of skin diseases, its antimicrobial activity has not been previously reported. Therefore, the effectiveness of this decoction was evaluated to determine its antimicrobial activity including resistant bacteria against skin pathogens and identify the antimicrobial active compound(s). The study of the decoction as well as its individual plant components is a timely study due to the rapidly increasing demand for detection of novel antimicrobial agents from plant sources, particularly for the treatment of infections caused by multiresistant antibiotic resistant pathogens.

The PPK decoction, methanol extracts of PPK decoction and extracts of individual plants were prepared. Preliminary phytochemical screening of PPK and individual plant extracts was carried out. To identify the active components, sequential extraction of PPK plants was carried out using hexane, dichloromethane (DCM), ethyl acetate, methanol and water, based on their polarity from less polar to high polar. Using TLC, chemical components of each extract was identified. For further identification, active extracts were fractionated using vacuum liquid chromatography. Nine fractions from the hexane extract and 8 fractions from the DCM extract were obtained. Extractions using non polar/less polar solvents suggested that the activity is associated with non/less polar compounds. As plant oils also contains similar compounds, oils were distilled from PPK and its component plants, using the Clevenger apparatus. GC-MS analysis of PPK oil confirmed the presence of compounds. Of the individual plants tested, *S. aromaticum* was identified as a highly active plant,

and its oil was subjected to column chromatography. Eugenol was isolated from the fraction by preparative TLC. Screening of extracts for antibacterial activity was performed using the cut well agar method for decoctions and methanol extracts, and disc diffusion method for plant fractions and oils. The minimum inhibitory concentration (MIC) of decoction and methanolic extracts was determined by the agar dilution method, for the decoctions and methanolic extracts, a broth dilution method was used for the fractions and oils. All experiments were conducted using standard aseptic techniques. Antibacterial activity of decoctions and methanolic extracts were evaluated against eight bacterial isolates (*Staphylococcus aureus* NCTC 6571, *Escherichia coli* NCTC 10418, *Pseudomonas aeruginosa* NCTC 10662 and five wild strains of Methicillin Resistant *Staphylococcus aureus* (MRSA). The oils of PPK and its component plants were evaluated against a spectrum of organisms including sensitive, standard organisms, Gram positive cocci, multi resistant Gram negative bacilli and 5 species of *Candida*. The test organisms were obtained from the Department of Microbiology,

Faculty of Medicine, University of Peradeniya.

Both, PPK decoction and methanol extracts of PPK plants inhibited the growth of methicillin sensitive as well as resistant *S. aureus*. Methanol extracts of the 12 plants showed inhibitory activity (ZOI 14-32 mm; MIC 0.2–15 mg/ml) against *S. aureus*. However, only 9 of the 13 plant decoctions showed activity against *S. aureus* at dilutions ranging from 1/5 to 1/160 (Table 1). Both, the decoction and methanolic extract of *S. aromaticum* inhibited *E. coli*. Methanolic extracts of *E. ribes* and *C. angustifolia* also inhibited *E. coli*. Both, the decoction and methanol extracts of *S. aromaticum* and *E. ribes* inhibited *P. aeruginosa*. Decoctions of *S. chinensis*, *I. aspalathoides* and *P. longum* and methanolic extract of *C. angustifolia* also inhibited *P. aeruginosa*. Preliminary phytochemical analysis of PPK (Fig. 2) and its individual plants showed that terpenoids are consistently present in 11 of the 12 plant species that showed inhibitory activity against *S. aureus* (Table 1).

The MICs of hexane, dichloromethane (DCM) and ethyl acetate extracts for *S. aureus* were less than

Table 1. Phytochemicals in PPK decoction, methanol extracts of PPK and extracts of individual plants with corresponding minimum inhibitory concentration (MIC)

Plants	Water soluble chemicals (decoction)			Methanol soluble chemicals					Sensitive <i>S. aureus</i>		MRSA	
	Terpenoids	tannins	saponin	Terpenoids	tannins	saponin	flavonoids	alkaloids	decoction	Methanolic extract MIC mg/mL	decoction	Methanolic extract MIC mg/mL
<i>S. aromaticum</i>	+	+	-	+	+	-	-	-	1/160	0.2	1/160	0.2
<i>E. ribes</i>	+	+	+	+	+	+	+	+	1/80	0.2	1/80	0.2
<i>C. angustifolia</i>	+	+	+	+	+	+	-	+	1/80	0.7	1/80	0.7
<i>P. longum</i>	+	-	+	+	-	+	-	+	1/40	5	1/40	5
<i>I. aspalathoides</i>	+	+	-	+	+	-	+	+	1/20	0.7	1/20	0.7
<i>M. fragrans leaf</i>	+	+	-	+	+	-	+	+	1/10	1	1/10	1
PPK	+	+	+	+	+	+	+	+	1/10	1.7	1/10	1.7
<i>S. chinensis</i>	+	-	+	+	-	+	+	+	1/5	3.2	1/5	3.2
<i>Z. officinale</i>	+	-	+	+	-	+	+	+	1/5	3.3	1/5	3.3
<i>P. nigrum</i>	+	-	-	-	-	-	-	+	1/5	4.5	-	4.5
<i>M. fragra. seed</i>	+	-	-	+	-	-	+	+	1/5	5	1/5	5
<i>E. litorale</i>	+	+	+	+	+	+	+	+	1/5	10	1/5	10
<i>A. tetraacantha</i>	-	-	+	-	-	+	-	+	-	10	-	15
<i>H. niger</i>	+	-	-	+	-	-	-	+	-	0.8	-	1.5

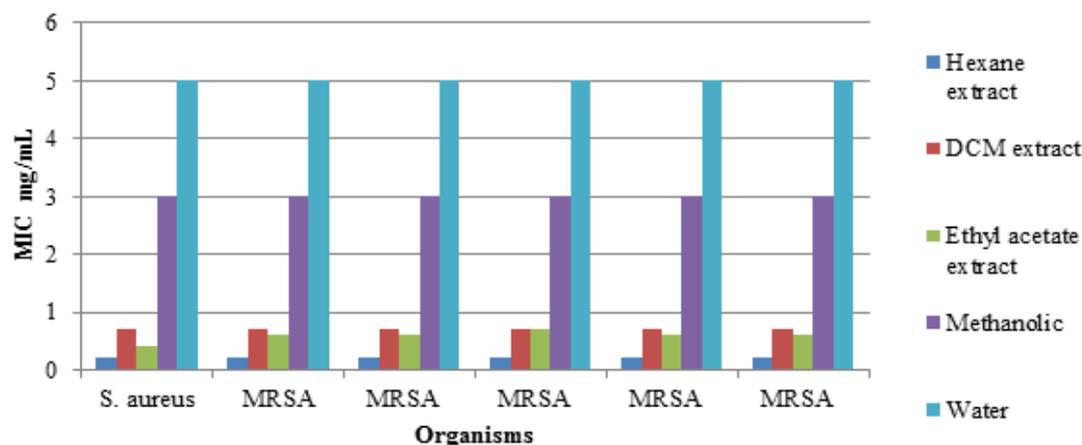


Figure 1. Minimum inhibitory concentration (MIC) (mg/mL) of the sequential extracts of PPK (Mean \pm SD)



Figure 2. Phytochemical screening of PPK

the MICs of the methanol and water extracts. Mean \pm SD of MICs of hexane, DCM and ethyl acetate extracts for all tested strains of *S. aureus* ranged from

0.2 \pm 0.0 to 0.7 \pm 0.3 mg mL⁻¹ suggesting that the active ingredients were less polar or medium polar compounds(Fig 1). Fractions Hexane 1, DCM 1, Hexane 4, Hexane 5 and DCM 5 possess inhibitory activity against *S. aureus* and *Candida* sp. H1 and D1 contain β -caryophyllene, β -pinene and limonene. Fraction H4, 5 and D5 contain several compounds including eugenol.

Oil obtained from PPK and 6 of the 12 individual plants were tested for antimicrobial activity against a wide spectrum of multidrug resistant (MDR) bacteria and *Candida* sp. *S. aromaticum* was the most active with MICs of 0.005 – 0.037 μ g/ml for *S. aureus*, 0.018–0.15 μ g/mL for MDR Gram negative Bacilli and 0.004 μ g/ml

Table 2. Minimum inhibitory concentration (MIC) of PPK oil, *S. aromaticum* oil and isolated Eugenol

Plant oils	Panel-1-Control strains			Panel-2-Gram positive cocci		Panel-3-MDR Gram negative bacilli				Pael-4- <i>Candida</i> sp
	<i>S. aureus</i> NCTC 6571	<i>E. coli</i> NCTC 10418	<i>Paeruginosa</i> NCTC 10662	5 MRSA strains	VRE	<i>K. pneumoniae</i>	<i>E. cloacae</i>	<i>Acinetobacter</i>	<i>Proteus</i>	<i>Candida</i> sp
PPK oil	0.05	0.32	0.64	0.160–0.320	0.640	0.310–1.250	0.310	0.150	0.310	0.075 – 0.310
<i>S. aromaticum</i> oil	0.005	0.018	0.31	0.018–0.037	0.075	0.037–0.075	0.150	0.018	0.037	0.004
Isolated eugenol	0.009	0.018	0.31	0.037–0.310	0.009	0.018	0.009	0.0045	0.009	0.002

for *Candida* sp (Table 2). Eugenol was identified as the main component in PPK and *S. aromaticum* oil, using GC-MS analysis and column chromatography.

The study infers that PPK decoction (Fig. 3) contains many compounds at very low Minimum inhibitory concentrations (MICs), important to human pathogens.



Figure 3. Parankipaddai Decoction

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Synthesis of hydroxyapatite nanomaterials for their potential use in fabrication of bone-implants



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In recent years, development of custom-made bone implants and synthesis of materials for bone grafting have become important areas in biomedical research.

A variety of nano-technological methods have been developed to synthesize nano-sized biomaterials, such as bio-ceramics, composites and polymers, to coat metallic bone implants to make them less corrosive, biocompatible and bio-functional. Furthermore, custom-made bone implants and their raw-materials are very expensive. They are imported to Sri Lanka at a high cost. Hence, production of low-cost raw-materials locally for bone-implants, would reduce medical expenses greatly. The research study explored novel methods to develop cost-effective, biocompatible prostheses and focused on (i) converting Eppawala apatite into high purity hydroxyapatite (HA) nanoparticles and other nano-composites (ii) synthesizing porous HA needed for drug delivery and bone-implants and (iii) coating of metallic surfaces of implants with thin films of HA and HA/TiO₂ through ASP technique.

Synthesis of HA Nanoparticles, HA/TiO₂ nanocomposites, HA/Polymer nanocomposites and Porous HA

Spherical and needle shaped HA nanoparticles were prepared using ammonium dihydrogen orthophosphate and calcium sucate. During the preparation of HA, the phosphate precursor was added to calcium sucate solution until Ca/P ratio 1:1.67 and a low, basic pH level was maintained. Formation of both spherical and needle shaped hydroxyapatite was identified by scanning electron microscopy (Fig. 1).

HA/TiO₂ nanocomposites

By modifying the above technique, sets of HA/TiO₂ nanocomposites samples were synthesized using TiO₂ solution and previously synthesized HA nanoparticles, under the hydrothermal synthesis method. The two HA/Polymer nanocomposites; HA/Poly(acrylate) and HA/PMMA (Poly(methyl methacrylate)) were synthesized using appropriate monomers. Hydrophobic HA/Stearic acid nanocomposites were synthesized using previously prepared HA nanoparticles and stearic acid.

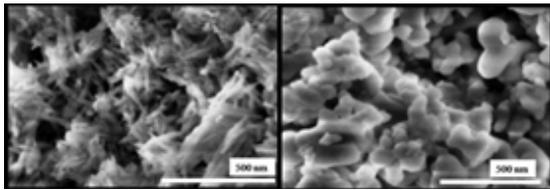


Figure 1. SEM images of spherical and needle HA

HA/Polymer nanocomposites and Porous HA

HA/polymer nanocomposites of acrylic polymer was prepared by mixing with the calcium sucrate solution, Ca^{2+} ions and chelating complexes. The Fig. 2 shows the SEM images of the prepared HA/PAA nanocomposites and images of the colloidal stability study. The HA/PMMA composite synthesized was a white coloured crystalline powder with fine particles. TEM images of prepared HA/PMMA are shown in Fig. 3. In order to prepare HA nanoparticles dispersed in PMMA polymer matrix, HA/ stearic acid composites was used.

Thereafter, HA nanocomposite dispersed PMMA polymer matrix was prepared to reduce usage of metallic implants. Nanoporous HA was synthesized on calcium carbonate template using surfactant under the calcium sucrate routes.

Conversion of Eppawala Apatite to Value-added Products

Eppawala apatite samples, collected from a phosphate deposit in Eppawala, Sri Lanka, were crushed and ground using a ball mil. Powdered

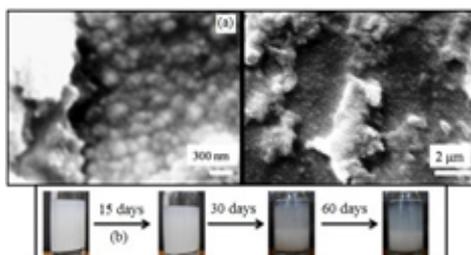


Figure 2. Images of HA/PAA (a) SEM and (b) Colloidal stability study

apatite samples were heated to produce calcined apatite and was dissolved in conc. HNO_3 acid.

The solution was heated with urea to start recrystallization *via* combustion and the resulting product was hydrothermally treated.

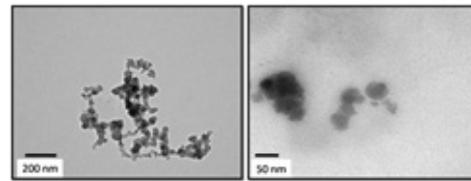


Figure 3. TEM images of HA/PMMA

Deposition of HA nanoparticles and HA/ TiO_2 nanocomposites on titanium metal surfaces via atomized spray pyrolysis technique (ASP)

Colloids of both HA nanoparticles and HA/ TiO_2 nanocomposites (HA nanomaterials) were prepared separately. Thereafter, clean Ti metal or Ti alloy pieces were heated and colloids were coated using the ASP technique.

Findings of the Study

There are several advantages of the preparation of calcium-based nanomaterials using calcium sucrate. From the calcium sucrate route, different types of morphologies of nanomaterials can be easily prepared starting from naturally occurring minerals.

The synthesized HA/PMMA composite was a white colored crystalline powder with fine particles. In order to prepare HA nanoparticles dispersed in PMMA polymer matrix, the prepared HA/ stearic acid composites has been successfully used due to their dispersing ability in MMA monomer than HA nanoparticles and the other HA nanocomposites. Hence, HA has been uniformly dispersed in PMMA polymer matrix after coating SA. The biocompatibility of composite materials also increases with increasing HA nanoparticles. Hence, the biocompatibility and mechanical properties of HA/SA composite dispersed PMMA polymer matrix would increase. For example, femoral stem of hip prosthesis can be prepared by incorporating a small metal rod during polymerization of HA/SA dispersed polymer matrix. The shape of the prosthesis is maintained using mechanical cutting process to suite the dimensions of the needy person, in order to produce a custom made prosthesis. Fig. 4 shows photographs of a metal rod incorporated HA dispersed PMMA matrix and HA-dispersed PMMA matrix. Hence, small implants, such as screws and pins, can be prepared using this PMMA matrix in order to reduce use of metals.

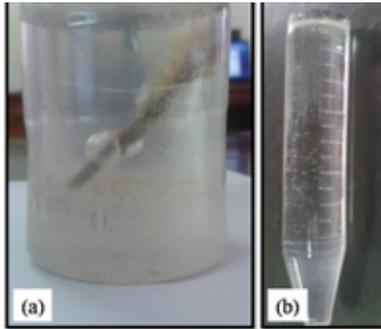


Figure 4. Images of (a) HA/ PMMA matrix with metal rod and (b) HA/PMMA matrix

During the preparation of nanoporous HA, CaCO_3 nanoparticles were formed after adding Na_2CO_3 to the calcium sucrate solution. Particle aggregation of CaCO_3 has been overcome by CTAC micelles. These CaCO_3 nanoparticles have been embedded to HA particles during the formation of HA. Voids of porous HA formed by removal of the internal porous nature of final HA product is clearly observed in TEM images (Fig. 5).

In the process of combustion of Eppawala apatite (EPAP), mixer of calcium phosphates has been produced from EPAP such as tricalcium phosphate and calcium pyrophosphate. Therefore, different hydrothermal conditions have been used to treat the synthesized product from EPAP under combustion method in order to produce pure HA. Purity of the final product was identified through EDX spectra. The EDX spectrum in Fig. 6a clearly indicates the presence of F and Cl in pure EPAP samples. However, F and Cl are removed during

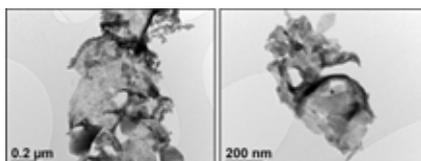


Figure 5. TEM images of nanoporous HA

the synthesis of HA nanoparticles which occurs during the process of combustion Fig. 6b. This is the importance of this conversion method of EPAP to the HA nanoparticles, because there is no report in literature on removal of incorporated F⁻ and Cl⁻ from EPAP. The unique feature of this novel process is that it enables the removal of harmful impurities from HA nanoparticles during the preparation of HA nanoparticles.

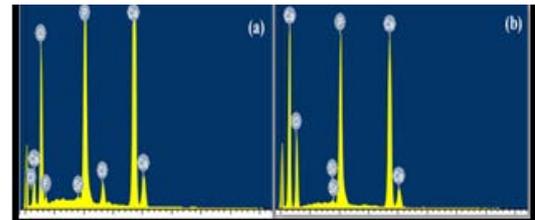


Figure 6. EDX spectra of (a) pure EPAP and (b) finally synthesized HA nanoparticles

In the ASP technique, first, the precursor solution is directed under high speed to an atomizing chamber using pressurized air which can make aerosol droplets. Separated, fine particles are deposited on the hot substrate surface through the spray nozzle, which is placed above the surface. The deposited species then, undergo pyrolysis to form nucleated species on the substrate surface, which will then grow into particles and get become attached to the surface of the substrate. HA nanoparticles and TiO_2 /HA nanocomposite coated Ti metals and Ti alloys are prepared using this technique. Corrosion rate of thin film coated such metals was drastically decreased with the thin film coating. Such a drastic reduction of the corrosion rate, is due to the increase of the barrier properties of the modified TiM, which has resulted from the formation of a pin hole-free thin film layer on Ti surfaces. Data given in Table 1 clearly indicate that the corrosion potential is shifting towards more anodic values.

Table 1. Electrochemical data pertinent to corrosion of bare and surface-modified Ti plates

Ti Sample	$E_{\text{corr}} / \text{mV}$	R_p / Ω	$J_{\text{corr}} / \mu\text{A cm}^{-2}$	$\text{CR} / \mu\text{m yr}^{-1}$
(a) Bare Ti plate	-566 ± 11	$(2.95 \pm 0.5) \times 10^4$	$(7.7 \pm 0.1) \times 10^{-1}$	9.001 ± 3
(b) HA coated Ti	-310 ± 12	$(1.87 \pm 0.2) \times 10^8$	$(1.1 \pm 0.8) \times 10^{-4}$	0.0014 ± 0.001
(c) TiO_2 /HA coated Ti	-245 ± 11	$(2.24 \pm 0.3) \times 10^7$	$(3.1 \pm 0.4) \times 10^{-3}$	0.10 ± 0.02
(a) Bare Ti alloy (TiA)	-334 ± 05	$(2.55 \pm 0.3) \times 10^6$	$(2.9 \pm 0.5) \times 10^{-2}$	1.0 ± 0.6
(c) TiO_2 /HA coated TiA	9 ± 0.8	$(1.75 \pm 0.8) \times 10^7$	$(4.9 \pm 0.3) \times 10^{-3}$	0.170 ± 0.02

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Atomized spray pyrolysis for fabrication of thin films and development of dye-sensitized solid-state solar cells



M.M.C.Sampath K. Ranasinghe obtained his BSc Special Degree in Physics in 2009 from the University of Peradeniya. Sampath embarked on research on fabrication of thin films and development of dye-sensitized solar cells on a local fellowship he received. His research study led to his PhD Degree from the PGIS. Currently, Sampath is conducting research at Toyota Technological Institute, Nagoya, Japan as a Postdoctoral Fellow. Email: mmcskrana@yahoo.com

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Energy production to fulfill the future demands of the people is a challenge faced by the present generation, world over. Electricity has become the main form of energy for large-scale industries and households as it can be converted into many other forms. Presently, the demand for the electricity is mainly fulfilled by non-renewable resources such as nuclear, coal power, and fossil fuels such as oil and gasses. To reach the goal of developing renewable energy sources, solar energy has been identified as the best source that could replace the centuries old hazardous technologies involving non-renewable resources. Dye-sensitized solar cell (DSC) is one of the primary types of solar cells with power conversion efficiency around 10%. Dye-sensitization and injection of carriers into semiconductor surfaces by photo-excited dye molecules was first observed by the Vogel in 1873. He observed that the dye coated silver halide grains enhanced the green and red response in photographic films. In dye-sensitized solar cells, thin porous semiconductor electrodes are used as anodes. To construct semiconductors, thin films spray pyrolysis technique is widely used. In this study, this technique was continuously used to fabricate different types of thin films. Generally, in spray pyrolysis deposition (SPD) technique, precursor solution is sprayed onto a heated substrate, where pyrolysis of the precursor takes place on the heated substrate. For the fabrication of porous films much lower temperatures are used while for dense films, much higher temperatures are used.

The atomized spray pyrolysis deposition (ASPD) technique was first reported in 2011, by a group that included a Sri Lankan chemist (H.M.N. Bandara). It is known to have a low cost of production, high reproducibility and uniformity and is capable of fabricating mesoporous semiconductor films where nano-size particle dispersions are used as the precursor solution. In this technique, kinetic energy of the high-pressure precursor is used to break the aggregated particles into

smaller units, which then come out as aerosols. The ASPD developed in 2011 suffered from many technical problems, limiting its application in the different fabrications due to contamination following corrosion of internal components. Therefore, it was essential to develop a full system with more versatile fabrication possibilities. Hence, the present study focused on the following specific objectives: (i) Development of atomized spray pyrolysis deposition (ASPD) instrument, (ii) Development of low-cost conducting glass using atomized spray pyrolysis technique, (iii) Development of dye-sensitized solid-state solar cells, (iv) Scaling-up of dye-sensitized solar cells, (v) Development of Pt-free counter electrode for the dye-sensitized solar cells and (vi) Development of low-cost counter electrode with comparable efficiency with high-cost Pt-based counter electrode. The following methodologies were used to meet the specific objectives of the study.

Development of atomized spray pyrolysis deposition (ASP) instrument

The ASPD system was developed with all the internal components of the atomizer constructed using Nylon and Teflon with more user-friendly designs. An aerosol transferring tube was introduced, which helps to transfer aerosols towards the slit-type nozzle from the hotplate above. Several electronic indicators to notify failures of the hotplate were introduced. To control the temperature of the purposely built hotplate, proportional integral derivative (PID), and overheat controller system was installed.

Development of low-cost conducting glass using atomized spray pyrolysis technique

For this, FTO thin films on low-cost soda lime glasses were fabricated using a novel ASPD technique. Also, SnCl₄ was used as the main Sn⁴⁺ source and NH₄F as the fluorine dopant source.

Development of dye-sensitized solid-state solar cells

Black dye (N749) and N719 cocktail dye system was used as the new co-sensitizing dye system.

The N749+N719 dye cocktail was tested with the earlier developed DSSC, based on n-type TiO₂ and p-type CuI. The DSSCs based on ZnO were constructed with the configuration of FTO/ZnO dense layer/ZnO porous layer/D149/ CuSCN/ Graphite/Pt-Cr mirror-FTO. Also, three types of SnO₂ composites, based on MgO, ZnO, and CaCO₃ electrodes were fabricated, and their performances were compared with the conductivity enhanced CuSCN to obtain high power conversion efficiencies (PCEs).

Scaling-up of dye-sensitized solar cells

Here, DSC modules based on TiO₂/N719/(I₃⁻/I⁻) electrolyte were fabricated.

Development of Pt-free counter electrode for the dye-sensitized solar cells

Activated carbon (AC)-fluorine doped tin oxide (FTO) composite was investigated, and the thin film was fabricated using spray pyrolysis deposition. The performance of the electrode was tested as a counter electrode in iodine electrolyte based dye-sensitized solar cells.

The constructed ASPD instrument was capable of performing different pyrolysis depositions in the temperature range of ambient to 550 °C. The atomizing system showed high chemical resistance with the Nylon and Teflon components. The performance of the ASPD system was further proved by the fabrication of FTO thin films at 450 °C. The FTO thin films fabricated using the new ASPD instrument, on the soda-lime glass substrate showed transmittance, electrical conductivity, electron mobility and the carrier density of, 85.2% at 660 nm, 1.71x10³ S cm⁻¹, 10.89 cm² V⁻¹ s⁻¹ and 9.797x 10²⁰ cm⁻³, respectively at room temperature. These measures showed the superiority of the FTO thin fabricated using the ASPD technique. The PCEs

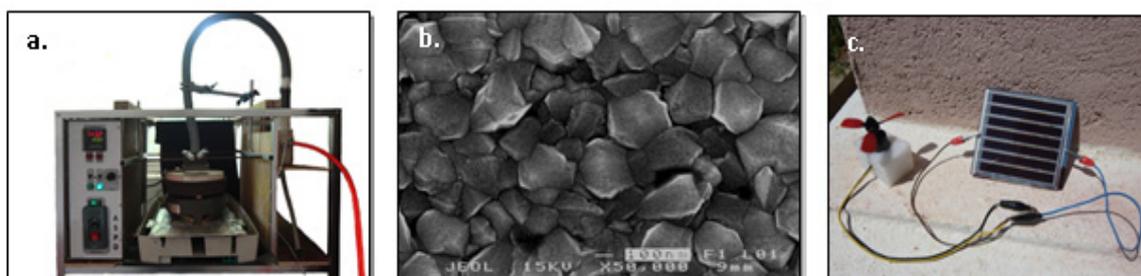


Figure 01. a) Atomized spray pyrolysis deposition instrument, b) SEM image of the FTO thin film fabricated using ASPD instrument, and c) 10 cm x 10 cm solar cell module developed in this study.

of the DSCs fabricated using these FTO substrates, and commercial FTO substrates were 10.4% and 9.1%, respectively. The PCE of the N719 and Black dye cocktail sensitized DSSC was 4.6% where individual N719 and Black dye-sensitized DSSCs showed 3.8% and 3.0%, respectively. The ZnO/CuSCN based DSSC showed a maximum PCE of 2.28% at a dense layer sheet resistance of 1500Ω and ZnO porous layer thickness of 9 μm. Furthermore, the SnO₂, SnO₂-MgO, SnO₂-ZnO, and SnO₂-CaCO₃ based DSSCs with CuSCN showed maximum PCEs of 0.32%, 2.82%, 2.38%, and 1.84%, respectively. The DSC with the counter electrode fabricated using activated carbon 0.400 g per 40.0 mL of FTO solution and sintered at 500 °C showed the highest PEC of 7.6%, where the Pt-Cr mirror-FTO type counter electrode showed 9.4%. The DSC modules of 10 cm x 10 cm gave over 0.700 mV of voltage and 0.5 A of current, under direct exposure to sunlight.

Possible applications of the atomized spray pyrolysis deposition system developed in this study are in thin film fabrication. Through the expansion of the developed technique, it is possible to fabricate conductive thin films and solar cells in Sri Lanka. Instruments and materials developed in this work are shown in Fig. 1.

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Distribution and ecology of land snails in the Knuckles Conservation Forest, Sri Lanka



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Sri Lanka is home to about 253 species of land snails of which 81% are endemic to the island. The pioneering studies on our land snails were carried out by the British, more than 100 years ago, who concentrated on the taxonomy and distribution of species. Studies by local researchers began in the late 1990s and were largely on the anatomy and morphology of land snails. Studies thereafter, led to the compiling of distribution records and preparation of a Checklist of Sri Lankan land snails. With the Darwin Initiative (UK), land snail diversity project in Sri Lanka (1999–2002) began ecological studies on land snails, of both native and introduced species. The focus of the present study was land snails in the Knuckles Conservation Forest (KCF), where their species richness, abundance and diversity were studied with reference to different vegetation types, environmental gradients, impact of forest degradation and human interference on the land snail community in the KCF. Knuckles Conservation Forest is located in the Knuckles mountaing range (Fig. 1).

The present study spanned over a period of three years during which land snails were sampled in 116 transects representing all climatic and vegetation types within the KCF (approximately 31,305 ha in extent), using a combination of timed direct search method and litter sieving method. The natural forest types sampled included intermediate zone forests (210–867 m above msl), sub montane forests (935–1331 m above msl), and montane forests (1350–1826 m above msl). The man-modified areas sampled included cardamom plantation (Fig. 1) in montane and sub montane zones, grasslands (Fig. 2) in the sub montane zone and the home gardens located deep inside the KCF.

The study resulted in the collection of a total of 5830 land snail shells and live snails and these belonged to 49 morpho-species representing 28 genera in 16 families. Among them were 29 endemic species and four endemic genera namely, *Ologospira*, *Ravana*, *Rathnadvipia*, and *Aulopoma*. Thirty seven species from the collection were positively identified, while undescribed taxa

Table 1. Contribution of endemic, native and exotic species to the total snail fauna in different vegetation types in the KCF.

	Elevation Range (m)	Number of Species			Total
		Endemic	Native	Exotic	
MF	1350 - 1620	21	14	-	35
SMF	935 - 1331	19	14	1	34
IMF	210 - 867	18	14	2	34
MC	1350 - 1826	13	11	1	25
SMC	1038 - 1257	16	11	1	28
SMGL	1010-1280	9	10	1	20
IMHG	233 - 741	9	7	3	19

MF – Montane Forests, SMF – Submontane Forests, IMF – Intermediate zone Forests, MC- Montane Cardamom plantations, SMC-Submontane Cardamom Plantations, SMGL- Sub Montanezone Grasslands., IMHG – Intermediate zone Home Gardens

accounted for 24% of the species (12) in eight genera. On average, each of the natural forests harboured 34 species of land snails (Table 1). Of the identified 37 species 15 species were restricted to the KFC, not being found elsewhere in Sri Lanka. They are, *Euplecta prestoni*, *E.colletti*, *E.layardi*, *Macrochlamys nepas*, *Macrochlamys woodiana*, *Mirusstalix*, *Thysanota elegans*, *Allopeas layardi*, *Idoartemon layardianus*, *Pupisoma longstaffae*, *Aulopoma grande*, *Cyclophorus ceylanicus*, *Japoniavesca*,

Thiobaldius bairdi and *Tortulo sanevilli*. It is of significance that 13 out of these 15 restricted species are endemic to Sri Lanka. Although the KFC snails exhibited a moderately high species richness, majority of the species were found in low abundance, a trait seen in land snail studies in other parts of the tropics. Individuals of three genera namely, *Euplecta*, *Glessula* and *Theobaldius*, were more common, occurring in more than 75% of the transects sampled.



Figure 1. Changes in the ground cover vegetation due to cardamom cultivation in the Knuckles Conservation Forest

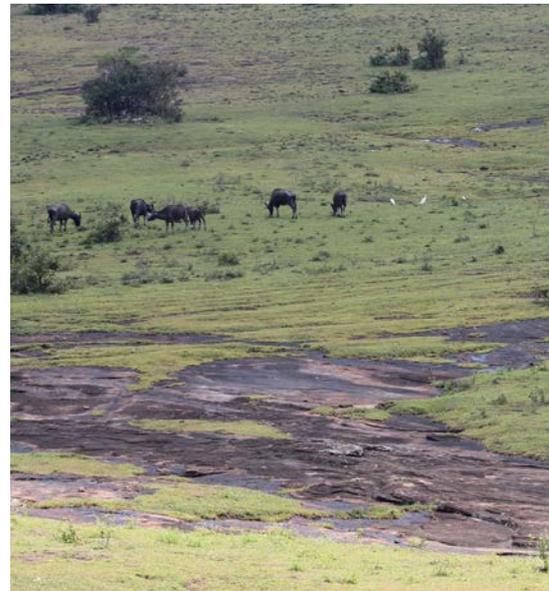


Figure 2. Pitawala Pattana, a grassland in the Knuckles Conservation Forest

Euplecta partita and *Theobaldiusannulatus* were the most abundant species encountered in the survey. A few species showed very restricted distribution. *Macrochlamys nepas*, and *Tortulosanevilli* were true montane species confined to montane forests above 1350 m altitude. *Euplecta prestoni* and *Thysanota elegans* were confined to the submontane forests, while *Euplectalayardi* was recorded from the Intermediate zone forests only. The land snail species, *Laeviculis altae*, which is a pest of plants, was recorded only from home gardens in the Intermediate zone.

The study highlights the rich endemic land snail fauna in the Knuckles Conservation Forest, of the 37 known species occurring in the conservation forest. Three environmental factors in the KFC, namely elevation, canopy height and canopy openness were found to contribute significantly to its land snail distribution and abundance. Land snail fauna were found to be fairly uniformly distributed across the three natural forest types in the KFC. Number of species in all three-forest types, irrespective of altitude and climate, was in the range of 34. This is in contrast to the altitudinal decline in species richness reported for land snail diversity elsewhere, mainly in the African tropics. The snail fauna in the man-influenced habitats was not equally distributed. The change in the species richness in human-influenced habitats may reflect human disturbance rather than the “effect of altitude, canopy height or canopy openness”.

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Chemistry and bioactivity of *Flacourtia inermis* fruits



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Research in natural products chemistry involves search for novel metabolites that have the potential to be used as pharmaceutical, nutraceutical, agrochemical and other products of economic interest. Many sources of bioactive metabolites remain unexplored for their biological and economic potential. These include common edible fruits which, if not harvested, marketed or consumed, are often discarded. One such fruit is, *Flacourtia inermis* Roxb. (F. Flacourtiaceae), locally known as “Lovi” (Fig. 1). Fruits are dark red when ripe with a sweet, sour and astringent taste. It is grown in home gardens and the excess crop is wasted. This study is an attempt to evaluate the economic potential of this fruit crop, which goes unused and wasted, every season. The study focused on important biological activities of the fruit metabolites and profiling the chemical composition, using LC-MS/MS analysis.

Extracts of *F. inermis* fruits were fractionated using chromatographic techniques and subjected to several bioassays in order to assess the nutritional and pharmacological value of the fruits and fruit extracts. Ripe, healthy fruits were used for the study and were extracted using sequential solvent extraction techniques. Fruits collected were cleaned and were checked for damage by any microbial attacks which would alter the chemical profile by changing the chemical nature and/or by contamination with microbial metabolites. Extraction was carried out by ultrasound sonication at room temperature and partitioning was used because fruits mostly contain thermolabile chemical compounds.

Fruits were blended and squeezed to separate the juice from the residue. The residue was subjected to ultrasound sonication using hexane, ethyl acetate (EtOAc) and methanol. Juice was partitioned with hexane, EtOAc and n-butanol. All the extracts were analyzed by thin layer chromatography. Hexane extracts and EtOAc extracts from both juice and residue were combined as they showed similar TLC patterns, then evaporated using a rotary evaporator at low temperatures and subjected to bioassays. The total polyphenol content and anthocyanin content of fresh fruits were determined. According to these results, anthocyanins contributed to approximately



Figure 1. Ripe fruits of *Flacourtia inermis*

10% of the total phenol content. The anthocyanin content in extracts is considered high compared to those reported for other berry type fruits.

The different extracts; EtOAc, methanol and n-butanol extracts of fruits, were subjected to the following assays to assess bioactivity; DPPH (2, 2'-diphenylpicrylhydrazyl) radical scavenging assay (Fig. 2), brine shrimp lethality assay (for cytotoxicity), lettuce seeds germination assay (for phytotoxicity) and TLC bioautography with *Cladosporium cladosporioides* (for antifungal activity). The same plant extracts were subjected to α -amylase inhibition assay (Fig. 3) against porcine pancreatic α -amylase enzyme, using starch as the

substrate and dinitrosalicylic acid as the indicator; lipase inhibition assay against *Candida rugosa* lipase enzyme, using *p*-nitrophenylbutyrate as the substrate and α -glucosidase inhibition bioassay against *Saccharomyces cerevisiae* α -glucosidase, using *p*-nitrophenyl- α -D-glucopyranoside as the substrate. Results of enzyme inhibition bioassays revealed significant activities against α -amylase, α -glucosidase and lipase enzymes.

The total polyphenol content of *F. inermis* fruits was determined as 1.28 g/100 g of fresh fruits by the Folin-Ciocalteu method using gallic acid as the standard. The anthocyanin content was found to be 0.107 g/100 g of fruits as cyanidin-3-glucoside equivalents, using a pH differential method. Polyphenols and flavonoids are among the natural active antidiabetic agents. These compounds have been reported to exert several biological effects, including inhibition of carbohydrate hydrolyzing enzymes, such as α -amylase and α -glucosidase enzymes. Polyphenolic compounds are able to inhibit the activity of digestive enzymes due to their ability to bind with proteins. The inhibitory activities of plant phytochemicals, including polyphenols, against carbohydrate hydrolyzing enzymes contribute to the lowering of postprandial hyperglycemia, which is useful in the management of diabetes. Polyphenolic compounds present in *F. inermis* fruit extracts are likely to have caused the inhibition of carbohydrate hydrolyzing enzymes observed during the current study.

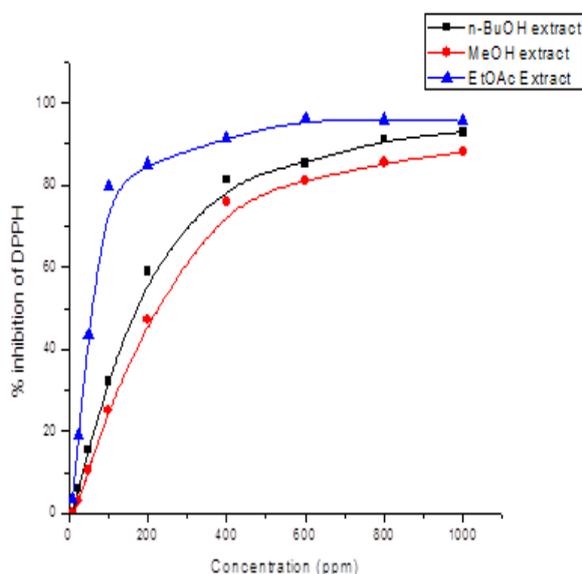


Figure 2. DPPH radical scavenging activity of crude extracts of *F. inermis*

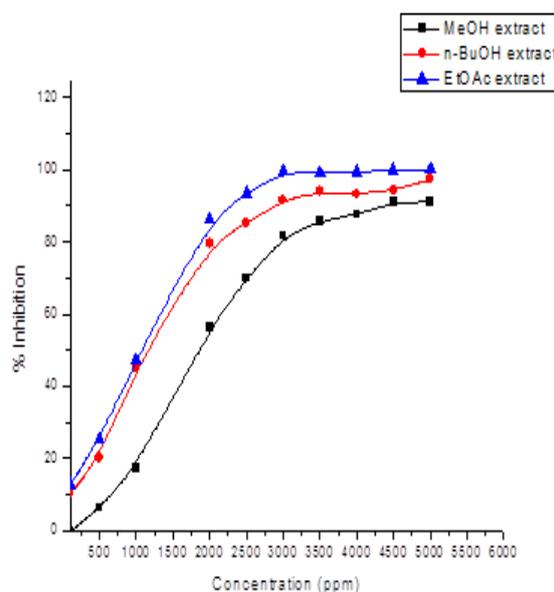


Figure 3. Percent inhibition of α -amylase enzyme activity of crude extracts of *F. inermis*

Each extract was tested for antioxidant activity against 2,2'-diphenylpicrylhydroxyl radical, antifungal activity against *Cladosporium cladosporioides*, cytotoxicity against nauplii of *Artemia salina* and phytotoxicity against *Lactuca sativa*. Activities exhibited by extracts were compared with positive controls. IC₅₀ values were calculated for dose-response relationships wherever possible. The EtOAc extract exhibited comparatively higher antioxidant activity than other extracts. Previous studies have reported the presence of antioxidant compounds in the fruits of *F. inermis*. Polyphenolic compounds including anthocyanins and chlorogenic acids are the antioxidant compounds reported from these fruits.

There was no observable antifungal activity against selected plant pathogens in any of the extracts. The n-butanol extract showed a comparatively high cytotoxicity against brine shrimp larvae. Some phenolic compounds and essential oils are cytotoxic against brine shrimp larvae. The high levels of phenolic acids in *F. inermis* extracts may account for the high cytotoxic activity. Brine shrimp lethality assay is considered to be a useful tool for preliminary assessment of toxicity. The results thus indicate that fruit extracts of *F. inermis* contain metabolites with potential cytotoxic activity.

The EtOAc extract was subjected to activity guided fractionation to isolate active compounds. The initial separation was carried out using normal phase gravity column chromatography using Hexane:EtOAc:MeOH and Hexane:CH₂Cl₂:MeOH solvent systems. Further purifications were carried out using size exclusion chromatography with Sephadex LH-20 and HPLC. This separation isolated one pure compound with remarkable α -amylase, α -glucosidase and lipase enzyme inhibition properties, and it was identified as S-malic acid; $[\alpha]_D$ -1.8 (c 0.38, H₂O) with ¹H NMR and ¹³C NMR spectral data. Malic acid showed high antioxidant activity and high inhibition activity of α -glucosidase, α -amylase and lipase enzymes. These findings suggest that malic acid is responsible for the antioxidant activity as well as the inhibitory activities of *F. inermis* fruit extracts against α -glucosidase, α -amylase and lipase enzymes.

Liquid Chromatography-Mass Spectroscopy or LC-MS/MS is a relatively novel tool for profiling chemical composition in a fast and effective manner. The principle of LC-MS/MS is based on the fragmentation of charged ions and the detection

of the resulting fragments. Thus, it has a much higher selectivity and sensitivity than LC-MS, and makes it possible to elucidate metabolite structures. During this study, LC-MS/MS analysis was used to identify chemical compounds present in *F.inermis*.

Methanol extracts of fresh fruits were subjected to LC-MS/MS analysis, and the data were analyzed using "Bruker Daltonics" data analysis software. Presence of different compounds can be identified from their base peaks at different retention times, and can be confirmed by analyzing their mass fragmentation patterns. LC-MS/MS analysis of the methanol extract of *F. inermis* fruits revealed the presence of many chlorogenic acids derivatives, including monocaffeoylquinic acids (Mr 354), dicaffeoylquinic acids (Mr 516), feruloylquinic acid (Mr 368), methylcaffeoylquinates (Mr 368), caffeoylshikimates (Mr 336), flavanoids and flavanoid-glycosides. CGAs were initially identified from their base peaks [M-H]⁻ at different retention times. Then MS² and MS³ fragments were identified at each retention time. In general, CGAs and their derivatives can be identified in an all tandem mass spectrum EIC (extracted ion chromatogram) by their unique fragments at m/z 173 and m/z 191. Analysis revealed the presence of a large array of chlorogenic acids (CGA) in *F. inermis* fruits. The term CGA is used to describe one important group of antioxidants, which are soluble esters formed by phenolic hydroxycinnamates with quinic acid, shikimic acid and their methylated/acylated derivatives. Some common hydroxycinnamates are *cis* and *trans* isomers of ferulic acid, *cis* and *trans* isomers of caffeic acid, *cis* and *trans* isomers of *p*-coumaric acid. CGAs can be divided into various groups depending on the identity, number, and position of the acyl moiety with the most common groups being *p*-coumaroylquinic acids, caffeoylquinic acids (CQAs), feruloylquinic acids (FQAs), caffeoylshikimic acids (CSA) and dicaffeoylquinic acids.

Five caffeoylquinic acids were identified and assigned using the hierarchical keys previously developed as the 3-*O*-caffeoylquinic acid, *cis*-3-*O*-caffeoylquinic acid, 4-*O*-caffeoylquinic acid, 5-*O*-caffeoylquinic acid and *cis* 5-*O*-caffeoylquinic acid. Three dicaffeoylquinic acids were detected and assigned using the hierarchical keys previously developed as 3, 4-*di-O*-caffeoylquinic acid, 3, 5-*di-O*-caffeoylquinic acid and 4, 5-*di-O*-caffeoylquinic acid. In addition, 3-feruloylquinic acid, methyl caffeoylquinates, caffeoyl-shikimates, flavonoids

and flavonoid-glycosides including Quercetin, Kaempferol and their glycosides were identified. The presence of S-malic acid, a combination of antioxidants, polyphenolic compounds including anthocyanins, chlorogenic acids and flavanoids indicated that *F. inermis* fruits, an under exploited fruit crop in Sri Lanka, has the potential to be used in health foods and nutritional supplements.

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Synthesis and characterization of novel electrolytes with possible applications in lithium batteries and dye sensitized solar cells



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The ever increasing demand for the energy production, storage and distribution has become the major goal and the challenge in the scientific world today. Solid state electrolytes are novel inventions that can be used in electrochemical devices. Among the various classes of solid state electrolytes, such as crystalline and composite electrolytes, polymer electrolytes (PEs) and ionically conducting glasses, PEs are identified as promising materials, especially due to their low weight, mechanical flexibility, possibility to obtain as thin films and low-cost. They are emerging as potential candidates for a variety of electrochemical devices such as rechargeable batteries, fuel cells, solar cells, super capacitors and electrochromic devices. The conductivity, voltage, capacity and recyclability of these devices need to be improved.

The objective of this study was to synthesize different types of novel electrolytes and characterize them by means of complex impedance spectroscopy, DC polarizing technique, Differential Scanning Calorimetry (DSC), optical micrography and Fourier Transform Infrared (FT-IR) spectroscopy. Ion interactions with polymer and solvent molecules were also analyzed in order to understand the conductivity mechanism of each system. The performance of these novel electrolytes in rechargeable batteries and dye sensitized solar cells (DSSCs) were also investigated. The solid polymer electrolytes ((PEO)_nPr₄N⁺I) with and without filler TiO₂ with different O:I molar ratios ($n = 5, 10, 20, 30, 40, 50, 60, 80$ and 90) were prepared by solvent casting technique. The different amounts of TiO₂ were added as a weight percentage of the polymer electrolyte. Plasticized gel polymer electrolytes (GPEs) were prepared by incorporating PAN and LiBF₄ into a mixture of EC/PC where the weight ratio of EC:PC was 1:1. Plasticized GPE was prepared, by dissolving the desired amount of LiBF₄ in a mixture of EC and PC, keeping the weight ratio at 1:1. The mixture was stirred until the salt was completely dissolved. To this solution, PAN was added and the mixture was heated at 140 °C, while magnetically stirring for

one hour. The resulting transparent homogeneous viscous solution was casted on to a Petri dish.

To prepare the fumed silica based tetraglyme/KI and ethylene glycol/KI polymer free gel electrolytes, the salt KI was dissolved in the solvent and magnetically stirred for 24 h until the salt completely dissolved. The composite gel was formed by adding the appropriate amount of fumed silica, to achieve a filler concentration of 10%wt

Poly(ethyleneoxide)(PEO), tetrapropylammonium iodide ($\text{Pr}_4\text{N}^+\text{I}^-$) and titania (TiO_2) based solid polymer electrolytes were synthesized to investigate the filler effect on iodide ion conductivity. Of the nine compositions synthesized, $(\text{PEO})_{10}\text{Pr}_4\text{N}^+\text{I}^-$ showed the highest ionic conductivity of $7.18 \times 10^{-8} \text{ S cm}^{-1}$ at 27 °C (Fig. 1). However, to investigate the filler effect on iodide (I^-) ion conductivity, solid polymer electrolyte $(\text{PEO})_{60}\text{Pr}_4\text{N}^+\text{I}^-$ with a moderate salt concentration was chosen to make the expected filler effect to be dominant. The addition of filler TiO_2 enhanced the amorphous nature of the electrolyte complex, according to DSC measurements, optical micrographs and FT-IR measurements. However, with the addition of filler there was no significant enhancement of ionic conductivity.

To prepare plasticized gel polymer electrolytes, polyacrylonitrile (PAN), lithium tetrafluoroborate (LiBF_4) with plasticizers ethylene carbonate (EC) and propylene carbonate (PC) were used. The highest ionic conductivity of $4.25 \times 10^{-3} \text{ S cm}^{-1}$ was obtained with $(\text{PAN})_5(\text{LiBF}_4)_{15}(\text{EC})_{40}(\text{PC})_{40}$. According to FT-IR analysis, Li^+ of LiBF_4 is predominantly interacting with EC rather than with PC due to its high dielectric constant. Lithium batteries were assembled using the electrolyte $(\text{PAN})_{10}(\text{LiBF}_4)_{10}(\text{EC})_{40}(\text{PC})_{40}$ with

and without filler TiO_2 . The lithium batteries prepared showed good recharging behaviours.

To prepare polymer free novel gel electrolytes, tetraethylene glycol dimethyl ether (tetraglyme)/ethylene glycol (EG), potassium iodide (KI) and fumed silica (SiO_2) were used.

Gel electrolytes, $(\text{tetraglyme})_{15}\text{KI}$ with 10% of wt. fumed SiO_2 and $(\text{EG})_{12}\text{KI}$ gel electrolyte with 10% of wt. fumed SiO_2 exhibited the highest ionic conductivities of $2.80 \times 10^{-3} \text{ S cm}^{-1}$ and $1.50 \times 10^{-2} \text{ S cm}^{-1}$ at 24 °C, respectively (Fig. 3.0). The FT-IR spectra showed that in both systems, the K^+ ions strongly interacted with the oxygen atoms of tetraglyme and ethylene glycol. Despite the higher ionic conductivities of $(\text{EG})_{12}\text{KI}$ with 10% of wt. fumed SiO_2 gel electrolyte system, DSSC fabricated using $(\text{EG})_{12}\text{KI}+10\%$ of wt. SiO_2 with 40:1 I^-/I_2 molar ratio gave relatively low energy conversion efficiency (1.35%) than the DSSC fabricated using $(\text{tetraglyme})_{15}\text{KI}+10\%$ of wt. SiO_2 with 10:1 I^-/I_2 molar ratio (3.54%).

Unlike in cation conducting PEs, in poly(ethylene oxide) (PEO), tetrapropylammonium iodide ($\text{Pr}_4\text{N}^+\text{I}^-$) and titania (TiO_2) based solid polymerelectrolytes, the anionic conductivity did not increase with the addition of the filler. However, according to the conductivity and DSC results, it can be concluded that the amorphous nature of the samples has been increased. Optical micrographs confirmed the amorphous nature, showing a reduction in size of the spherulites, with the addition of the filler.

In the PAN/ LiBF_4 /EC/PCplasticized gel polymer electrolyte system, a maximum conductivity of $4.25 \times 10^{-3} \text{ S cm}^{-1}$ was obtained for the GPE sample containing the highest salt concentration (composition a)(Fig. 2). However, its mechanical

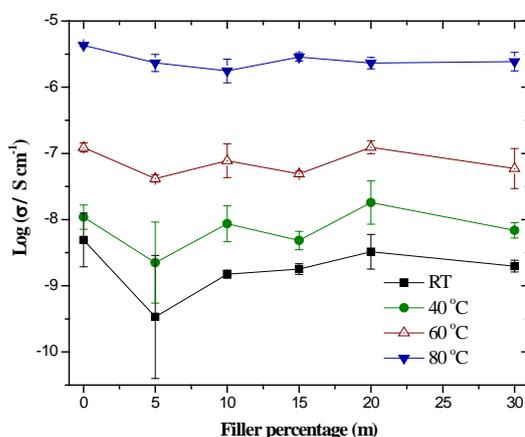


Figure 1. The variation of ionic conductivity as a function of filler percentage for $(\text{PEO})_{60}\text{Pr}_4\text{N}^+\text{I}^- + m\%$ wt. TiO_2 ($m = 0, 5, 10, 20$ and 30) at different temperatures.

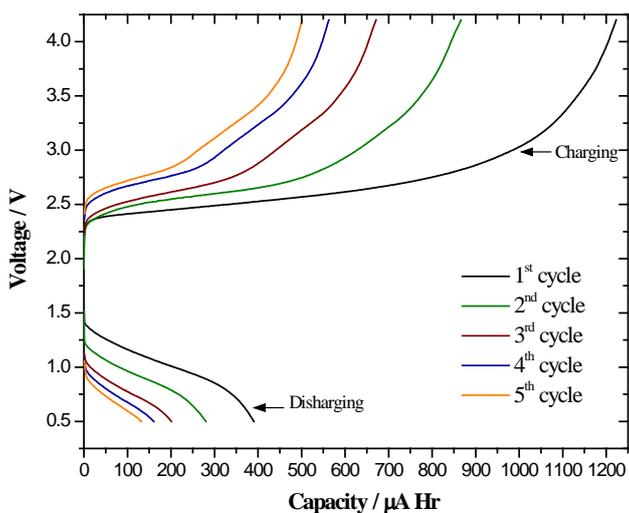


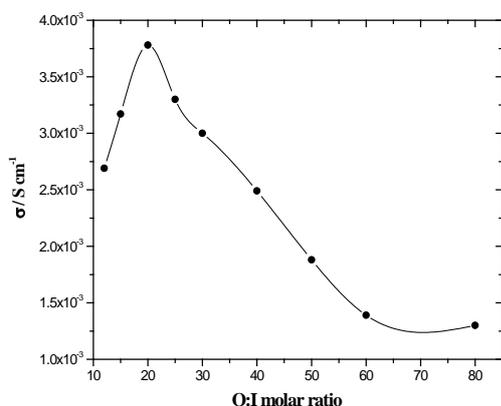
Figure 2. The graph of voltage vs. capacity of the cell $\text{LiMn}_2\text{O}_4/\text{GPE}/\text{graphite}$, fabricated using GPE with $(\text{PAN})_{10}(\text{LiBF}_4)_{10}(\text{EC})_4(\text{PC})_{40}$ (with 10% wt TiO_2).

stability was poor compared to the composition b. The filler (TiO_2) improved the mechanical properties of the GPEs without significantly reducing the ionic conductivity. The GPEs (with and without filler) prepared were predominantly ionic conductors with negligibly small electronic conductivity. According to FT-IR spectra of GPEs, the changes in the band shape, band width and vibrational frequencies indicated that there are strong interactions of Li^+ ions of dissociated LiBF_4 with the plasticizer molecules.

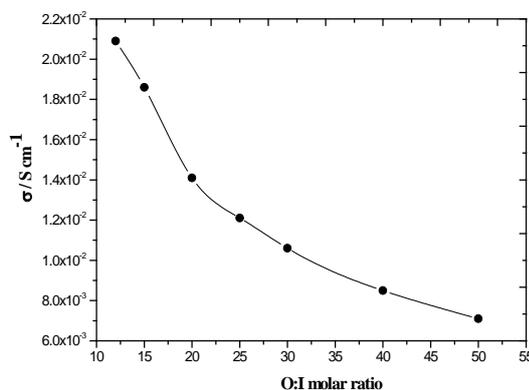
Mixing of fumed silica, gave gel electrolytes more stability compared to liquid electrolytes, leading to successful fabrication. The GEs

prepared were predominantly ionic conductors.

The addition of fumed silica to liquid electrolyte formed a gel without a significant drop of ionic conductivity. The highest ionic conductivity of $2.80 \times 10^{-3} \text{ S cm}^{-1}$ was observed for the tetraglyme based GE of O:I molar ratio of 15:1 with 10 wt.% fumed silica (Fig. 3). For ethyleneglycol based GE system, the highest ionic conductivity of $2.01 \times 10^{-2} \text{ S cm}^{-1}$ was observed with the electrolyte at 12:1 molar ratio. According to the FT-IR spectra of liquid electrolytes, the changes in the band shape and vibrational frequencies indicated that there are strong interactions of K^+ ions of dissociated KI with the ether oxygens of tetraglyme and ethylene glycol



(a)



(b)

Figure 3. (a) The ionic conductivities of the $(\text{tetraglyme})_n \text{KI}$ liquid electrolytes with different O:I molar ratios, (b) The ionic conductivities of the $(\text{EG})_n \text{KI}$ liquid electrolytes with different O:I molar ratios.

molecules. The three gel electrolytes synthesized and characterized in this study show promising ionic conductivities, making them suitable electrolytes for Li ion batteries and dye sensitized solar cells.

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Crime data analysis framework with geographical information support for intelligence led policing



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Currently, the Sri Lanka Police records and investigates crime data manually. Crime data are entered into books resulting in large stacks of books. This has made maintenance and analysis of crime data difficult and inefficient. Only records of the last five years are kept due to storage limitations. This system has many drawbacks; (i) allows criminals to conduct a series of crimes not being noted for the previously convicted crimes, (ii) use of Bristol boards for crime maps and crime clocks (Fig. 1) and (iii) crime information being made available only to local police stations. Several proprietary crime data analysis systems are available in foreign Police Departments, such as, CopLINK, TAS (Timeline Analysis System) and AICAMS project. They are custom made for legislative authorities in their own countries and the systems are not accessible outside the respective authorities. Most of these systems lack a complete knowledge base and are very costly. Also, the customization of the software to suit the current manual process adopted by Sri Lanka Police is very tedious. This research study attempted to introduce a crime analysis framework with a collection of crime investigation algorithms/methods to efficiently deal with problems associated with the current manual system.

The broad focus of the research study was to introduce a robust framework to the Sri Lanka Police with a database, which can facilitate a comprehensive environment to accommodate data mining techniques along with crime recoding and investigation functionalities. Thereafter, to follow a progressive procedure to introduce crime investigation techniques and data mining techniques. The specific objectives that were addressed were (i) Requirement elicitation (Jointly with the Sri Lanka Police) to identify functional and non-functional requirements, (ii) Formulation of a data model for the criminal data analysis framework, (iii) Analyzing and designing a framework for criminal data analysis, (iv) Integrating a crime mapping system with the crime data manipulation system, (v) Evaluating and validating the implemented crime data manipulation system, (vi) Developing an efficient real-time modus operandi detection method suitable for a large database system, (vii) Evaluating and testing the modus operandi

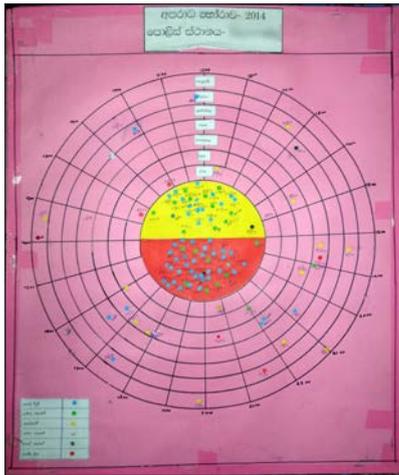


Figure 1. A manual crime clock

detection algorithm, (viii) Developing a suitable method to detect crime hotspots in GIS maps in large databases, (ix) Developing software tools to analyze crime clock data and (x) Develop a software tool for GIS vector data analysis using topological analysis and machine learning methods. This module will be useful in finding shortest paths from one location to another.

The proposed solution named ‘Sri Lanka Crime Investigation Decision Support System’ (SL-CIDSS) is a web-based intelligent crime analysis system enriched with Geographic Information Systems (GIS) support. Fig. 2 shows the GIS module of SL-CIDSS. The system comprises data mining tools in support of efficient information extraction and crime analysis from historical and current crime data.

SL-CIDSS was tested for efficiency by analyzing the query processing time data, and compared with its earlier version SL-SecureNet. After the framework was fine-tuned with the crime data handling logics, the basic analysis methods, such as crime clock, crime comparison and crime pattern visualizer were incorporated. These three tools solely run upon crime frequency data which are directly retrieved through the framework. Thereafter, an efficient method of linking crime and criminals was deployed. A novel method of using J48 classification algorithm in finding the nearest police station to a particular crime location in a map (NPSD – Nearest Police Station Detection), was proposed. The method classifies the coordinates of a particular location in search, to a predefined class which in turn is a police station. As the Sri Lanka Police currently does not have a predefined method of assigning sufficient security to different areas, a GIS-based hotspot detection algorithm was implemented. This algorithm logically provides a quantitative analysis of how much security is needed at a particular location.

The ‘Sri Lanka Crime Investigation Decision Support System’ (SL-CIDSS) is enriched with data mining techniques, Geographical Information Systems (GIS) and a layered architecture. The element interaction of SL-CIDSS is shown on Fig. 2. Tools, such as Crime clock, Crime comparison, Crime pattern visualization, Modus operandi analysis, Nearest police station detection (NPSD) and Hotspot detection are integrated to the framework. The crime clock provides the crime frequencies for the 24 h, which can be viewed for a custom date range. The crime comparison tool also provides a graphical interpretation of the

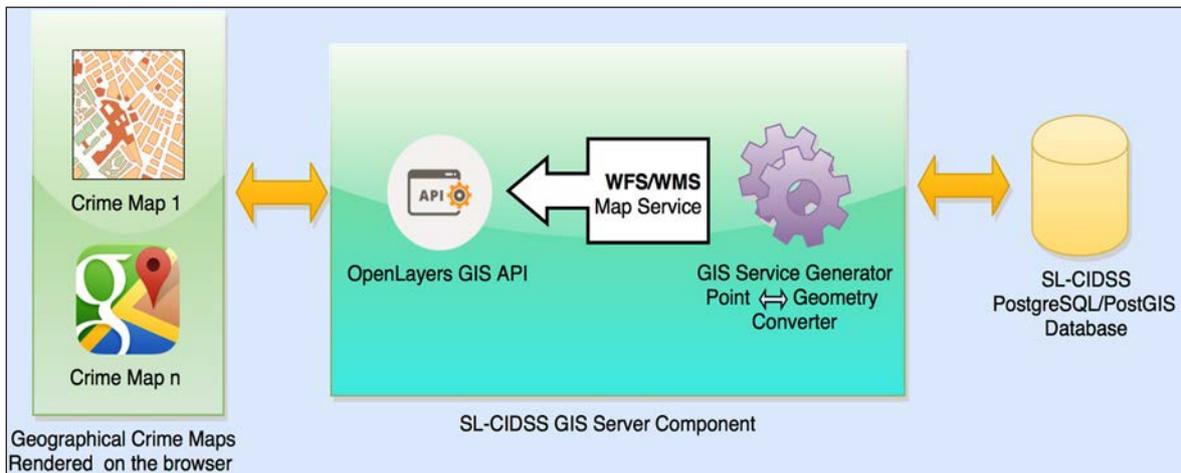


Figure 2. GIS module of SL-CIDSS

crime frequencies with the aid of a pie chart. Crime pattern visualization uses a line chart to compare the crime trends over a pre-defined time span.

Fuzzy based binary feature profiling for modus operandi (MO) analysis algorithm presents a method which is focused on identifying the perpetual modi operandi of criminals by analyzing their previous convictions. The method involves a fuzzy inference system to generate a similarity score in order to identify suspected criminals. Receiver operating characteristic (ROC) analysis was performed to justify the validity and generalizability of the fuzzy based binary feature profiling for modus operandi analysis algorithm. The method shows an Area under curve (AUC) of 0.6937 to an increased AUC of 0.9749 with the introduction of over sampling to the dataset. Also, the comparisons showed that the proposed MO analysis method performs comparatively well with Logistic Regression, J48 Decision Tree, Radial Basis Function Network, Multi-Layer Perceptron and Naive Bayes Classifier.

In the NPSD tool, J48 classification algorithm was trained in classifying the geographical coordinates to a set

of predefined classes that are police stations distributed on a map of interest. Results showed that, NPSD has an average accuracy of 98.21%. Hotspot detection helps to identify the areas with higher crime densities than others, so that security can be arranged accordingly. The data points are clustered by a non-hierarchical algorithm which uses a predefined grid and a threshold value. Each cluster is denoted by a colour and a radius according to the magnitude of the cluster.

According to the test results ,the proposed framework identified criminals very successfully with a high accuracy and an acceptable speed which consumed an average time of 51 sec to retrieve a query of 277,854 criminal records. Furthermore, the use of layered architecture makes the framework maintainable and extendible, and GIS capabilities assure the assistance on GIS analysis which can be very important in crime investigations.

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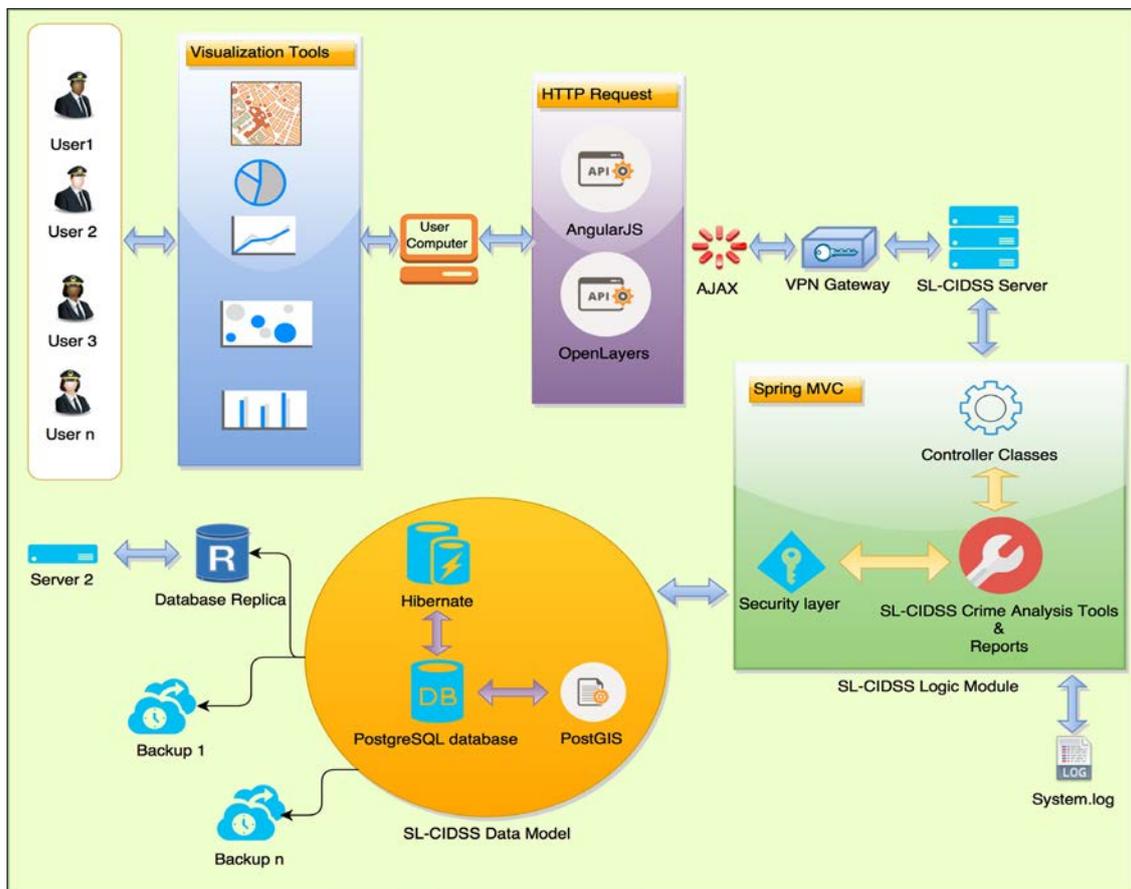


Figure 3. Element interactions of SL-CIDSS

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Short term rainfall forecasting using artificial intelligence techniques



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Predicting the amount of precipitation in a certain location for a future time period is referred to as quantitative precipitation forecasting. Precipitation can either be due to rainfall, snow, sleet or hail. In Sri Lanka, precipitation occurs due to rainfall. Many human activities are dependent on rainfall thus, the necessity to forecast rainfall. To obtain rainfall forecasts, data on previous and current atmospheric conditions are manipulated using scientific methods with technological assistance.

Rainfall forecasts are used as an input in many decision support systems and early warning systems because natural disasters like floods, draughts and landslides are highly dependent on rainfall occurrences. A survey conducted out by Climate Change Secretariat of the Ministry of Environment, Sri Lanka in 2009 has identified urban development, human settlements, water, agriculture, fisheries, health, biodiversity and ecosystem services sectors to be highly vulnerable to floods, draughts and landslides. An accurate rainfall forecasting system can help in making the correct decisions in these sectors and provide early warnings in case of natural disasters, to minimize the damages and inconveniences faced by the public. Producing an accurate quantitative rainfall forecast is a very difficult task due to the complexity of rainfall process.

An artificial neural network (ANN) is an information processing paradigm that uses the information processing principles of biological nervous systems to process information. It is composed of a large number of highly interconnected processing elements (neurones) working together to solve specific problems. ANNs are used in applications, such as pattern recognition and data classification. They are trained for a specific application through a learning process by adjusting synaptic weight. ANNs can be used to extract patterns and detect trends that are too complex to be noticed by either humans or other computer techniques due to their ability to derive meaning from complicated or imprecise data. A trained neural network can be thought of as an “expert” in the category of information it has been given to analyse. This expert can then be used to provide projections

given new situations of interest. Thus, an ANN will be ideal to handle the complex and imprecise weather data and provide a precise forecast of rainfall.

ANN is a forecasting tool that can handle complicated data efficiently. Different types of ANNs exhibit different advantages. A collection of a finite number of ANNs trained for the same task is called an ensemble neural network. In an ensemble separate ANNs are trained individually, and then their outputs are combined. The accuracy of a prediction depends on the ANN's generalization ability. It has been shown that the generalization ability of an ensemble is significantly increased than of a single classifier, which was also confirmed by our past studies. Many studies have been carried out to find methods to further increase the generalization ability of the ensembles. Their results showed that the generalization ability of an ANN ensemble increases when only a few number of ANNs are included in the ANNs ensemble rather than all available ANN and also when the member ANN is accurate and diverse. Diversity of ANNs is that their errors represent different regions in the input space. Accuracy and diversity are two conflicting conditions that have to be balanced carefully to achieve good performance. The performance of an ensemble also depends on the way the members are connected. This study presents two methods for selecting the most suitable members for an ANN ensemble. The main

objective of this study was to research and develop an ANN model that can be used to forecast the short-term rainfall accurately for a given area in Sri Lanka.

As the study area, Colombo was selected. It is located in the Western coast of Sri Lanka on North latitude $6^{\circ} 55'$ and East longitude $79^{\circ} 52'$, in the Wet Zone with an annual rainfall of about 240 cm. Daily rainfall data of for Colombo, over a period of 41 years (1961-2001) was collected from the Department of Meteorology, Sri Lanka, as the output of the ANN models. The NCEP_1961-2001 dataset derived from the NCEP reanalysis was used as the input of the ANN models. The data set contained 26 variables of daily observed data over a period of 41 years (1961 - 2001) Creating an ANN ensemble included three steps; creating the set of ANN, selecting the suitable ANN from the trained set and combining the selected ANN to obtain the output. There are many different methods to perform each of these steps and each step will contribute to the generalization ability and of the ANN ensemble. ANN pool was created using a set of Generalized Regression Neural Networks (GRNN) trained with different training data.

The **ENN-K Model** Diversity can be described as the difference in ANN error when the same input data is given. To identify the most diverse ANN, the ANNs were clustered according to their RMSE on

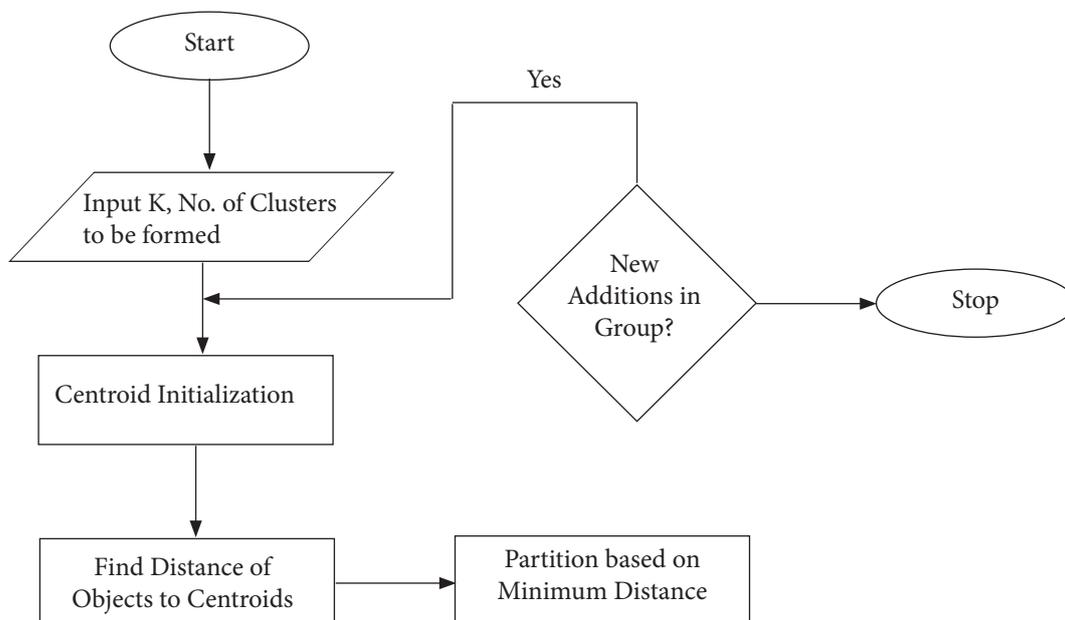


Figure 1. The K-Means clustering algorithm

validation data using k-means clustering algorithm. The errors of ANN in the same cluster are similar and the errors of ANN in different clusters are different. As a result ANN in each cluster is diverse from the ANN in other clusters. The algorithm for creating ENN-K model is shown in Fig. 1.

ENN-GA Model

In this model the ANN for the ensemble were selected using a binary GA to minimize the RMSE of the ensemble. The algorithm for creating ENN-GA model is described here (Fig. 2).

The best result was obtained by ENN-GA model with 14 GRNN in the ensemble with the chromosome

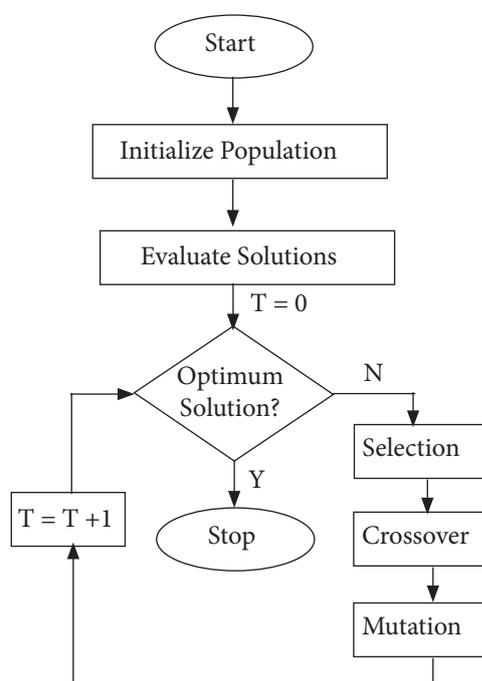


Figure 2. Basic genetic algorithm.

size 150. Fig 3. shows the RMSE obtained for ENN-K and ENN-GA for different numbers of ANNs in the ensemble. Table 1. shows the performance of ENN-GA for the three chromosome lengths.

A comparison of the performance of each model by the Root Mean Square Error (RMSE), Mean Absolute Error (MAE) and the R^2 are shown in Table 2. The study introduced two methods; ENN-K and ENN-GA methods to select the most appropriate members for a neural network ensemble developed for rainfall forecasting. Colombo, Sri Lanka was chosen as the study area for this study and the implemented ANN models were trained, validated and tested using daily observed weather data of 41 years. According to the findings, ENN-GA is able to create the best performing ensemble with a 14 member GRNN and a RMSE of 7.30. The ENN-GA was able to reduce the difference between the actual rainfall and the forecasted value considerably, for higher rainfall, resulting in a lower RMSE.

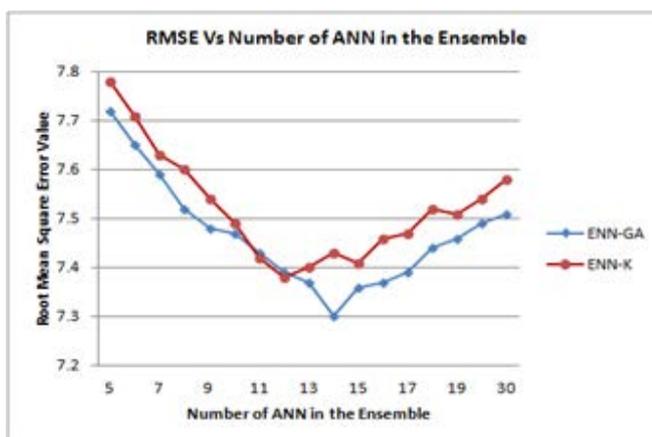


Figure 3. Performance of ENN-K and ENN-GA models for different sizes of ensembles.

Table 1. Genetic algorithm performance for different chromosome lengths.

Parameter	Chromosome Length		
	100	150	200
Number of ANNs in best performing ensemble	10	14	14
RMSE of the best performing ensemble	746	730	733
Number of generations for convergence	378	291	342

Table 2. Performance of the best ensemble in each model.

Model	No. of ANN	RMSE	MAE	R^2	Testing Time (sec)
ENN-K	12	7.38	4.32	0.673	94
ENN-GA	14	7.30	3.53	0.723	87

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Chemistry and bioactivity of secondary metabolites isolated from seeds of *Pouteria campechiana* and the associated endophytic fungus



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The genus *Pouteria* (F.Sapotaceae) is represented by a group of plants consisting of 9 sections and 325 species. Many of the species are used in folk medicine for several purposes. However, the chemical composition and biological properties are known only in a few species. Triterpenes, alcohols, acids, esters and flavonoids are the main chemical constituents of this plant genus. Species of *Pouteria* have been evaluated as a source of enzyme to be used as a synthesis reagent as well as for its biological activity. *Pouteria campechiana*, locally called 'lavulu' and commonly known as canistel or egg fruit, is an underutilized fruit tree native to Central America. It is grown in home gardens in Sri Lanka and other South Asian countries. It is cultivated in the Wet and Intermediate Zones of Sri Lanka. Studies have reported the presence of carotenoids such as neoxanthin, β -carotene, β -cryptoxanthin and violaxanthin in fruits of *Pouteria campechiana*. A high, total carotenoid content (1.9 to 23.5 mg g⁻¹ dry weight) has been reported from plants, but there are no reports on the seeds of *Pouteria campechiana*. Trichocomaceae is a family of fungi (Order Eurotiales and Class Eurotiomycetes) with several genera important to both industry and medicine, and includes the genus *Talaromyces*. This genus with more than 42 species is important in the food industry, medicinal drug industry and as bio control agents of pests. The present study focused on two aspects; chemistry and bioactivity of secondary metabolites in seeds of *Pouteria campechiana* and the endophytic fungus *Talaromyces purpurogenus* (syn. *Penicillium purpurogenum*) associated with *P. campechiana* seeds.

Seeds from ripe fruits *P. campechiana* were removed in a laminar flow. Inner and outer parts of seeds were triple sterilized, dried, placed on PDA petri dishes and incubated at room temperature for 3-4 days. The fungi that emerged were sub cultured to obtain a pure culture of the fungus. The endophytic fungus was isolated and initially identified as *Penicillium purpurogenum* by its microscopic and morphological characteristics.

Thereafter, by sequence analysis of ITS region of rDNA gene, the endophytic fungus was identified as *Talaromyces purpurogenus*. According to National Centre for Biotechnology Information (NCBI), *Penicillium purpurogenum* is a synonym of *Talaromyces purpurogenus*. A pure culture of the fungus in PDA media was inoculated in PDB media, on a large scale (20 x 1L conical flasks, each containing 400 mL of PDB medium) and allowed to stand at room temperature for 10 days, and thereafter incubated while shaking (100 rpm) every other day for another 18 days. After 28 days the PDB media was filtered. The filtrate was partitioned with EtOAc/H₂O to give EtOAc extract. The residue was crushed into small pieces and sequentially extracted into EtOAc and MeOH using sonicator to obtain n EtOAc and MeOH extracts. All three extracts (EtOAc filtrate, EtOAc residue and MeOH residue) were subjected to bioassays for (i) antifungal activity, (ii) antioxidant activity, (iii) cytotoxicity, (iv) phytotoxicity and (v) α -Amylase inhibition assay. The EtOAc extracts were chromatographed over silica gel (*n*-hexane-EtOAc-MeOH or *n*-hexane-CH₂Cl₂-MeOH) and Sephadex LH 20 (MeOH) and preparative thin layer chromatography, that furnished four compounds.

Preliminary investigations indicated the EtOAc extract of *P. campechiana* seeds were strongly active

for antifungal, antioxidant, phytotoxic and cytotoxic bioassays. The *n*-BuOH extract of seeds also showed significant positive responses to antioxidant and cytotoxic bioassays. Chromatographic separation of EtOAc and *n*-BuOH extracts of seeds furnished five compounds; Taxifolin (**116**), Gallicocatechin (**117**), Quercetin (**118**), 4-hydroxyacetophenone (**119**) and a glycerol ester of an unsaturated fatty acid (356 g mol⁻¹, **120**) (Fig. 1). The fatty acid was found to have antifungal activity against *Cladosporium cladosporioides* while Taxifolin, Gallicocatechin, Quercetin and 4-hydroxyacetophenone were found to be antioxidant. The fatty acid was significantly active against brine shrimps and 4-hydroxyacetophenone was moderately active. The fatty acid was strongly phytotoxic active while 4-hydroxyacetophenone was moderately active.

All three extracts (EtOAc filtrate, EtOAc residue and MeOH residue) from the endophyte were found to be antioxidant and phytotoxic while only EtOAc extracts were toxic to brine shrimp. The EtOAc extracts of endophyte *T. purpurogenus* furnished four compounds; 4-hydroxyacetophenone (**119**), Tyrosol (**121**), Taloroconvolutin A (**122**) and a new secondary metabolite; furanone analogue of Taloroconvolutin A (**123**). Taloroconvolutin A was significantly active against brine shrimp.

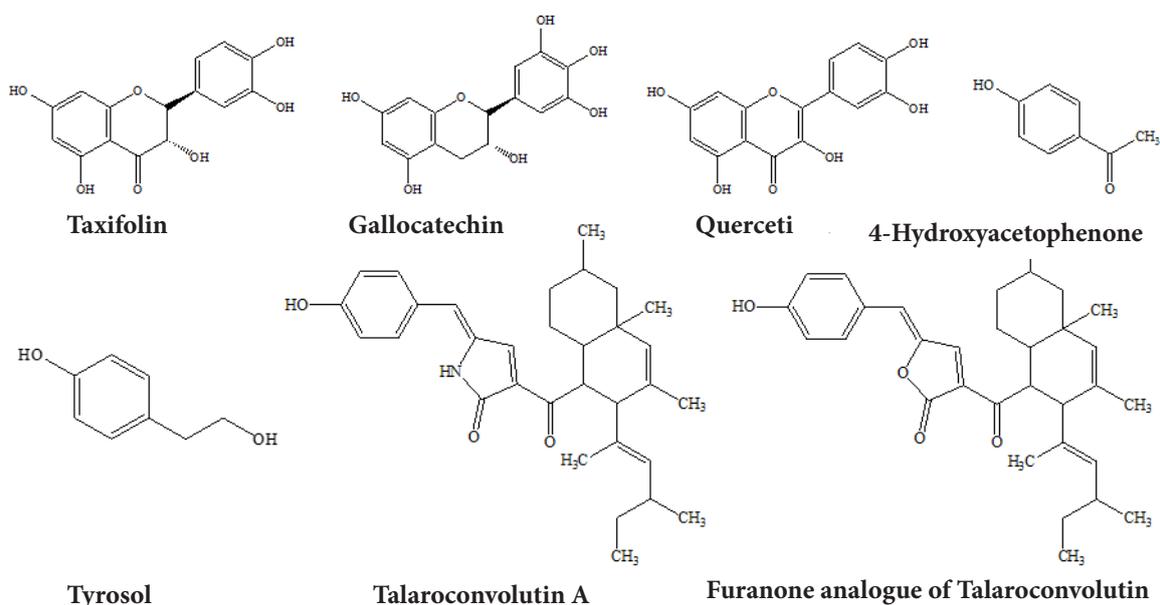


Figure 1. The compounds obtained from chromatographic separation of seed extracts of *P. campechiana*

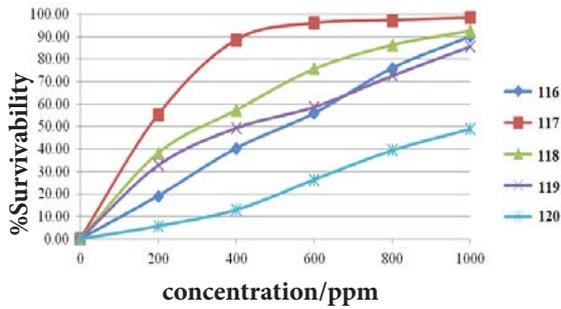


Figure 2. Antioxidant activity of pure compounds in seed extracts of *P. campechiana*

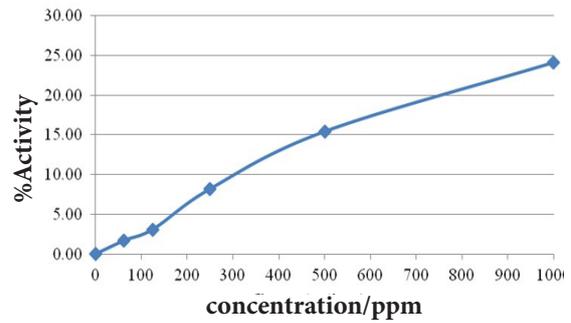


Figure 3. Antioxidant activity of Talaroconvolutin A

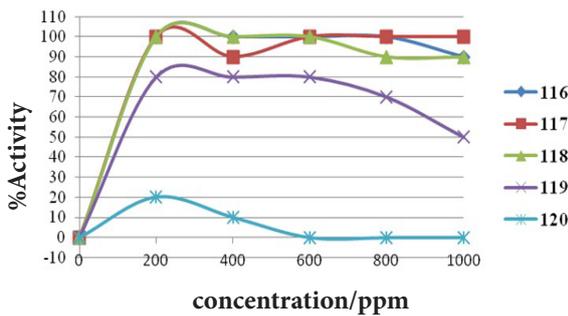


Figure 4. Percentage survival of brine shrimps in pure compounds of seed extracts of *P. campechiana*

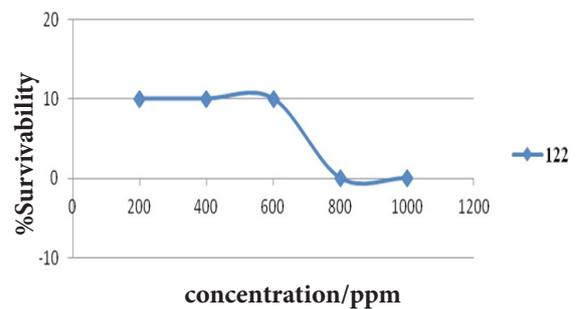


Figure 5. Percentage survival of brine shrimps in Talaroconvolutin A

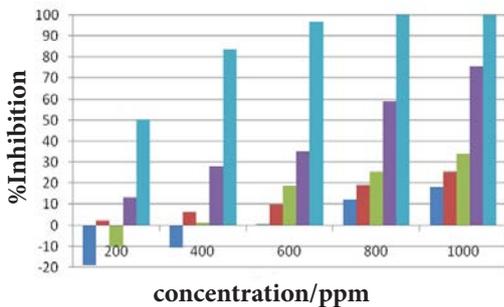


Figure 6. Percentage shoot inhibition by pure compounds of seed extracts of *P. campechiana*

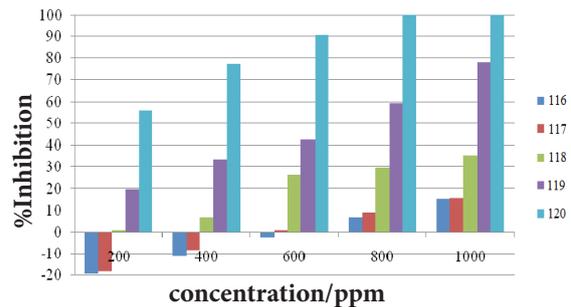


Figure 7. Percentage root inhibition by pure compounds of seed extracts of *P. campechiana*

Source of Funding

National Institute of Fundamental Studies, Kandy, Sri Lanka

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Ecology of the serpentine vegetation at Ussangoda, Sri Lanka



Y. A. S. Samithri graduated in 2006 with a BSc Special Degree in Botany from the University of Peradeniya. Samithri joined the The Open University of Sri Lanka, Nupe, Matara as a Lecturer in 2009. She embarked on postgraduate research on serpentine vegetation obtaining her MPhil Degree from the PGIS. Her research interests are in post-harvest technology of fruits and vegetables.
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Serpentine deposits are found in most parts of the world and have attracted the attention of scientists due to their unique soil characteristics. Serpentine soils are derived from weathering process of ultramafic rocks which have a high concentration of magnesium (Mg), iron (Fe) and, heavy metals such as nickel (Ni), manganese (Mn), chromium (Cr), cobalt (Co). These rocks are deficient in several essential plant nutrients such as calcium (Ca), nitrogen (N), potassium (K), phosphorous (P). Further, they exhibit an imbalance between calcium to magnesium ratio. The species composition, structure, endemism and the diversity of the vegetation in serpentine deposits are different from those in the surrounding areas. In addition, most plants on serpentine ecosystems have evolved to survive in metal rich soils to tolerate and even hyper-accumulate heavy metals in their tissues.

There are a few serpentine out crops in Sri Lanka at Ussangoda, Indikolapelassa, Ginigalpelessa, Yodhagannawa, Katupotha and Rupaha. However, information on the vegetation of serpentine soils is rather scant. The Ussangoda serpentine deposit in the Hambanthota district of Sri Lanka, is the only site located on the coastal area of the country.

The study was conducted (i) to determine the diversity and spatial patterns of serpentine vegetation in Ussanagoda, (ii) to determine levels of selected physico-chemical parameters (soil pH, moisture, total organic carbon and Ca, Mg, Ni and Fe content) in soil therein and (iii) to determine the levels of aforementioned metal ions in selected plants at the serpentine.

The study area selected was a relatively flat plain having herbaceous prostrate plants, scattered trees and shrubs in small thickets ('forest islands') of varying sizes. Herbaceous vegetation in 21 experimental plots (25 × 25 m²) established at randomly chosen points was enumerated. Further, the frequency of occurrence of all plant species in 'forest islands' was recorded and analyzed to determine the diversity of vegetation in relation to the size of 'forest island'.

To determine soil chemical parameters, samples were collected from three randomly laid quadrates (1 m²) in each of the experimental plots. To determine metal (Ca, Mg, Ni and Fe) accumulation in plants, plants from the 'serpentine plains' and 'forest islands' were used.

A total of 67 plant species in 61 genera and 30 families were identified from the study area. Among them, were herbaceous plants (18 spp.) in seventeen genera and 11 families. The plant species diversity was higher in 'forest islands' than in the extensive serpentine plain that encompasses 99% of the serpentine land area. The vegetation in 'forest islands' comprised 49 plant species in 44 genera and 27 families that included nearly threatened (4 spp.) and vulnerable(2 spp.) plant species.

Only 2 endemic plant species were present in the Ussangoda study area. The species richness among 'forest islands' was similar, irrespective of their size. However, no endemic or threatened plants were found in smaller 'forest islands' indicating the necessity to protect the remaining large 'forest islands' from anthropogenic activities. If the size of forest islands were to decrease, they would not support endemic and threatened plants.

According to Ordination analyses (Fig. 1), there is no variation in species composition and abundance of plants over the area, in the serpentine plains. The

species composition and abundances of plants did not show any relation to the examined soil parameters. However, certain herbaceous species showed a significant correlation with certain soil parameters.

Several serpentine plants appeared to be well adapted (eco-physiologically) to its metal rich soil. Thus, the six species; *Hybanthus enneaspermus*, *Euphorbia thymefolia*, *Evolvulus alsinoides*, *Vernonia cineria*, *Olax imbricata* and *Flacourtia indica* were found to be hyper accumulators of Ni and accumulators of some other elements such as Fe, Cr, Pb, Na, Cr, Co and Mg. Hence, these plant species could be used in phytoremediation, a green technology to clean up toxic metals in soil. Further, this study clearly identified the characteristic features and ecological significance of Ussangoda (Fig. 2) serpentine site and thereby, provided important information for the conservation and management of this distinctive ecosystem.



Figure 2. Serpentine outcrop in Ussangoda, Sri Lanka

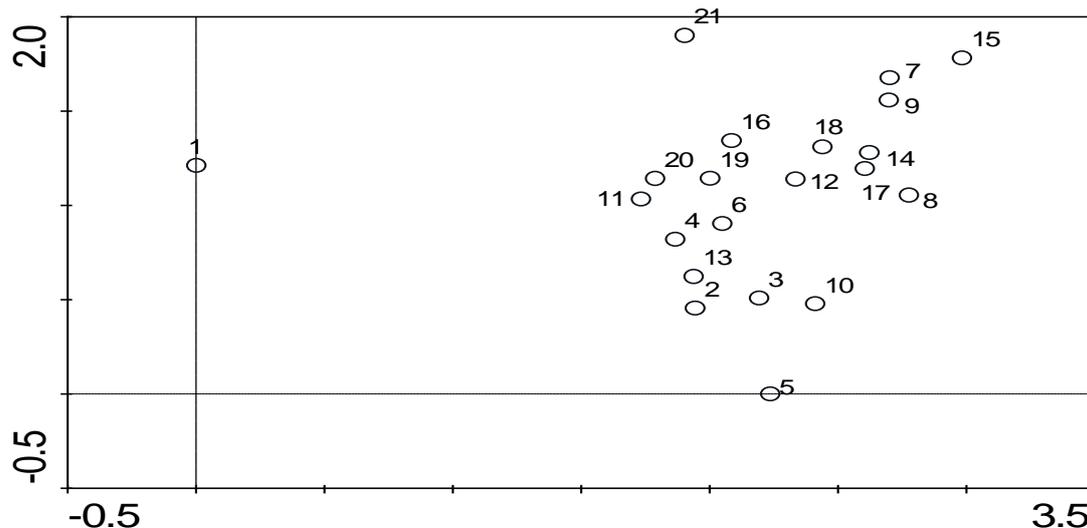


Figure 1. Scattergram of DCA of herbaceous species abundance data from 21 sampled plots produced by CANOCO version 4.5.

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Sri Lanka
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Kinetics and equilibrium aspects of interaction of heavy metal ions and peat in natural and modified forms



M.U. Sampath Wickramasooriya graduated from the University of Peradeniya in 2011 with a BSc Degree in Physical Sciences. Thereafter, sampath embarked on postgraduate research in the field of Environmental Analytical Chemistry obtaining his MPhil Degree from the PGIS. On successful completion of his MPhil Degree in 2016, he joined Natures Beauty Creations Limited as a Research and Development Chemist.

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Sri Lanka is moving towards rapid industrialization, and many industries require water in different stages of production. Consequently, large volumes of industrial effluents are released to the environment, mainly to water resources. Effluents released from many industries, such as electroplating, leather tanning, and metal finishing and processing, are contaminated with heavy metal ions and their derivatives. As heavy metals do not undergo biodegradation or decomposition, they would subsequently enter the food chain, causing harmful effects to human health, such as cancers, liver failure and renal failure. Treatment of industrial effluents to remove heavy metals before they are disposed into water resources is required to mitigate environmental pollution problems and health hazards. It would be more desirable to use natural, environmentally friendly and low-cost substances to remove such toxic substances. There are reports on the potential ability of natural substances to remove pollutants, although detailed investigation on peat available in Sri Lanka for the removal of heavy metal ions from the environment has not been reported.

The main peat deposit in Sri Lanka is in the Muthurajawela area of the Western Province. The large laterite-bound Muthurajawela peat (MP) deposit has been studied for its distribution of metals, and engineering aspects, such as permeability, elasticity and strength. Most metals, in particular, Be, Zr, Li and the majority of the transition series metals, are found to exhibit a strong positive correlation with K, Al, Fe and Mn. A noteworthy feature is the poor correlation of the metals with organic carbon, in contrast to a strong correlation with elemental components of clay. The laterites found around the peat deposit appear to be the possible source materials for the metals present. Further, use of MP as a trapping material for Cu(II), heavy metal – fulvic acid complexation reactions in simulated environmental systems, and metal organic interactions in aquatic systems have also been reported, promoting the necessity to investigate MP for various applications.

The overall objective of the study was to investigate how heavy metal ions interact with peat. Toxic metal ions which are commonly found in industrial effluents, such as Cu(II), Cr(III) and Cr(VI), were used in the study. Suitability of employing peat, in raw or processed form, for the removal of heavy metal ions from wastewater was also an objective.

The following procedure was followed to fulfill the above objectives: (i) Sampling and conditioning of peat, (ii) Bulk characterization of peat: Qualitative and quantitative determination of metallic constituents and different elements present in each sample type; Identification of organic functionalities, (iii) Surface characterization of peat: Surface titrations and scanning electron microscopy (SEM) of selected peat samples, (iv) Optimization of experimental parameters for interaction of each metal ion with each type of peat sample, (v) Investigation of the effect of processing (modification) of peat on heavy metal ion-peat interactions, (vi) Investigation of the effect of interferents [e.g.: Cd^{2+} , Cu^{2+} , Al^{3+}] on the extent of interaction, (vii) Investigation of equilibrium properties and adsorption isotherms for each metal ion, (viii) Investigation of kinetics of sorption for each metal ion, (ix) Investigation of the mechanism of metal ion-peat interactions and (x) Extension of this methodology to treat synthetic industrial effluents.

Representative samples of MP were taken at the $7^{\circ} 4' 32.5 \text{ N}$, $79^{\circ} 51' 58.2 \text{ E}$ geographical location (Fig. 1). All samples were allowed to air-dry for a few

weeks until there was no further mass loss. Randomly obtained air-dried samples of MP were ground to obtain particles of required diameters, followed by manual homogenization. These samples were thermally treated for 4 h at free determined temperatures from 100°C to 700°C with 100°C intervals. All batch (static) experiments were conducted with peat particles of diameter (d) < 1.0 mm, while glass columns used for dynamic experiments were packed with larger particles of diameters $1.0 \text{ mm} < d < 2.0 \text{ mm}$ to prevent clogging of the column with peat and to increase the flow rate of metal ion solutions as needed. Powdered peat particles of $d < 0.10$ mm followed by thorough mixing to obtain fairly uniform particle sizes were used to conduct XRD, XRF, FTIR and SEM experiments.

Standard solutions of Cr(III), Cr(VI) and Cu(II) were prepared using analytical grade $\text{Cr}(\text{NO}_3)_3$, $\text{K}_2\text{Cr}_2\text{O}_7$ and CuSO_4 , respectively. Solutions of different pH values were prepared using HCl and NaOH. For interference studies, solutions containing Na^+ , NO_3^- , Al^{3+} , PO_4^{3-} and SO_4^{2-} ions were prepared using NaNO_3 , $\text{Al}_2(\text{SO}_4)_3$ and KH_2PO_4 . Solutions of NaNO_3 of concentrations ranging from 0.05 mol dm^{-3} to 5.00 mol dm^{-3} were used for the investigation of the effect of ionic strength on the removal of Cu(II), Cr(VI) and Cr(III) by natural peat and peat thermally treated at 200°C . A sample of commercial humic acid (HA) was used to compare its spectral features with those of HA extracted from MP (M-HA), which was also used in Cu(II) removal experiments.



Figure 1. Sampling site at Muthurajawela peat deposit

The main results obtained from this research study are:

1. The organic content of MP in its natural form is 37%. Further, MP consists of Cl⁻, NO₃⁻, SO₄²⁻, PO₄³⁻ and Zn(II) in its aqueous leachates.
2. The point of zero charge (pH_{pzc}) of MP in its natural form, thermally treated at 200 °C and thermally treated at 400 °C are 3.8, 4.2 and 8.0, respectively.
3. Optimum conditions for metal ion removal are:

Metal ion	Shaking time/h	Setting time/h	Treatment emperature /°C
Cu(II)	2.5	1.0	200
Cr(VI)	2.0	1.5	200
Cr(III)	3.0	2.0	200

4. The extent of removal of Cu(II) by M-HA is significantly higher than that of MP, indicating that humic acid present in peat interacts with Cu(II) providing a significant contribution toward Cu(II) removal. It is therefore suggested that phenolic and carboxylic acid functionalities form complexes with Cu(II).
5. Isotherm studies show multi-layer coverage of all three metal ions on MP treated at 200 °C.
6. Thermodynamics of Cu(II) removal is an endothermic, spontaneous process.
7. Interferents, such as Na⁺, NO₃⁻, PO₄³⁻, SO₄²⁻, Al(III) and Cd(II), do not show considerable effect on the removal of Cr(III), Cr(VI) and Cu(II) by MP.

This research study involved the use of a wide spectrum of instrumental techniques in order to solve an environmentally significant problem, the removal of Cu(II) and chromium species from water using a low-cost, environmentally friendly adsorbent. The main contribution of this study to scientific advancement is the completion of many aspects of a pollutant/adsorbent system, namely, parameter optimization, surface characterization, equilibrium and kinetics, and dynamic studies for Cu(II), Cr(III) and Cr(VI) removal.

Under optimized conditions, the most efficient removal of Cu(II), Cr(III) and Cr(VI) from synthetic industrial effluents prepared in the laboratory was obtained. Further, surface characterization, and equilibrium and kinetics studies were investigated in order to understand the mechanism of interaction of chromium species with fired Muthurajawela peat.

Source of Funding

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Aloe vera gel incorporated beverages: Development, chemical characterization and evaluation on newly diagnosed Type 2 diabetes mellitus



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Aloe vera leaf gel is rich in polysaccharides, including acemannan (partially acetylated glucomannans), the primary active substance in the parenchyma, is reported to have many health beneficial effects including anti-diabetic effects. *Aloe vera* gel is extensively used as a functional ingredient in food and beverage production. However, aloin, an anthrone compound, found just beneath the skin of the leaves has been proven to possess laxative activity and bitterness. Preservation of health beneficial components including acemannan and removal of aloin are the major challenges in the beverage processor. Most methods employed for the processing result in end products, which do not preserve considerable amounts of acemannan and complete elimination of aloin and *vice versa*. The project addressed several aspects relating to the preparation of the aloe based ready-to-serve (RTS) beverage, namely, to determine the best process for its preparation with aloe gel particles, to develop it further to produce an anti-diabetic formula, to test its shelf-life and effect on Type 2 diabetes mellitus (T2DM) and to quantify analyse for the major constituents, aloin and acemannan.

For the removal of aloin from aloe gel, a water treatment method was established, and the residual aloin was determined by visible spectrophotometry. To obtain the desired mouth-feel of the beverage, the product was treated with pectinase enzyme at 50 °C for 20 min. Aloe gel pieces in the beverage was dispersed using Gellan gum. The effect of two pasteurization conditions; heating at 65 °C for 15 min (LTLT) and at 85-95 °C (HTST) for 1-2 min; on retention of acemannan was investigated. The other processing parameters for producing a normal formula of *Aloe vera* gel incorporated ready-to-serve beverage were established. Fig. 1 shows the process flow diagram for the development of the normal formula of *Aloe vera* RTS beverage. The normal formula was further developed as an anti-diabetic formula with sucralose as the sweetener followed by HTST pasteurization. The shelf-lives of the products were assessed based on physico-chemical, microbiological and sensory

qualities at 35 ± 2 °C. Fig. 2 shows the *Aloe vera* RTS beverage preparations developed according to normal formula and anti-diabetic formula.

An open-label, randomized clinical trial was conducted for three months to evaluate the effect of the anti-diabetic formula of *Aloe vera* RTS beverage on T2DM. (Ethical clearance was obtained from the Ethics Review Committee, Faculty of Medicine, University of Peradeniya.) Newly diagnosed T2DM patients who provided written consent to participate in the study were randomly assigned to receive orally 180 cm³ *Aloe vera* RTS beverage (10% w/v aloe gel) daily (90 cm³ twice a day) or anti-diabetic medication (metformin or/and sulfonyl urea). The total study population was 14 randomized patients (seven/ group). In each month venous fasting blood glucose (FBG), capillary fasting plasma glucose (FPG) and capillary postprandial blood glucose (PPBG) were determined. HbA1c was tested before and after the treatment period, using HPLC method (at Nawaloka Metropolis, Kandy). Capillary blood glucose was analyzed using glucometers (Free Style Optium). Other blood parameters were determined using an automated Chemistry Analyser (ELECYS 2010- Venus Diagnostic Services, Kandy).

Aloin content, in gel pieces treated with water for its removal, was 0.73 ± 0.12 mg dm⁻³ and was within safety limits for oral consumption. Acemannan content was considerably higher on HTST pasteurization followed by pectinase treatment. Acemannan contents of HTST and LTLT pasteurized normal formula of *Aloe vera* beverage were 857.5 ± 246.4 and 638.5 ± 62.4 mg dm⁻³, respectively. The pH, titratable acidity, total soluble solids and turbidity of the HTST pasteurized normal formula of *Aloe vera* beverage were 3.44 ± 0.02 , $0.42\pm 0.03\%$ w/v (citric acid), 9.33 ± 0.23 °Brix and 19.9 ± 1.1 NTU, respectively. The pH, titratable acidity, turbidity, total soluble solid and acemannan content of the anti-diabetic formula of *Aloe vera* beverage were 2.82 ± 0.05 , $0.52\pm 0.02\%$ w/v (as citric acid), 16.3 ± 0.9 NTU, 0.0 ± 0.0 °Brix and 2427 ± 115 mgdm⁻³, respectively.

The shelf-life of HTST pasteurized normal formula and HTST pasteurized anti-diabetic formula of *Aloe vera* beverage packaged in glass bottles was nine months at 27 ± 2 °C with acceptable physico-chemical, microbiological and sensory qualities. Clinical trials revealed that *Aloe vera* ready-to-serve beverage can lower venous FPG, capillary FBG, capillary PPBG and HbA1c without any documented short and medium term side effects. Significant differences in capillary FBG, venous FPG, capillary PPBG, and HbA1c were observed between *Aloe vera* group and anti-diabetic medication group. Capillary FBG was reduced from 234 ± 68 to 145 ± 42 mg dl⁻¹ and capillary PPBG was reduced from 230 ± 10 to 217 ± 92 mg dl⁻¹ in *Aloe vera* group at the end of the study. Venous FPG was reduced from 211 ± 71 to 157 ± 85 mg dl⁻¹ in *Aloe vera* group. HbA1c of *Aloe vera* group was reduced from 10.5 ± 1.7 to $8.2\pm 2.1\%$. As there was a considerable reduction of HbA1c with

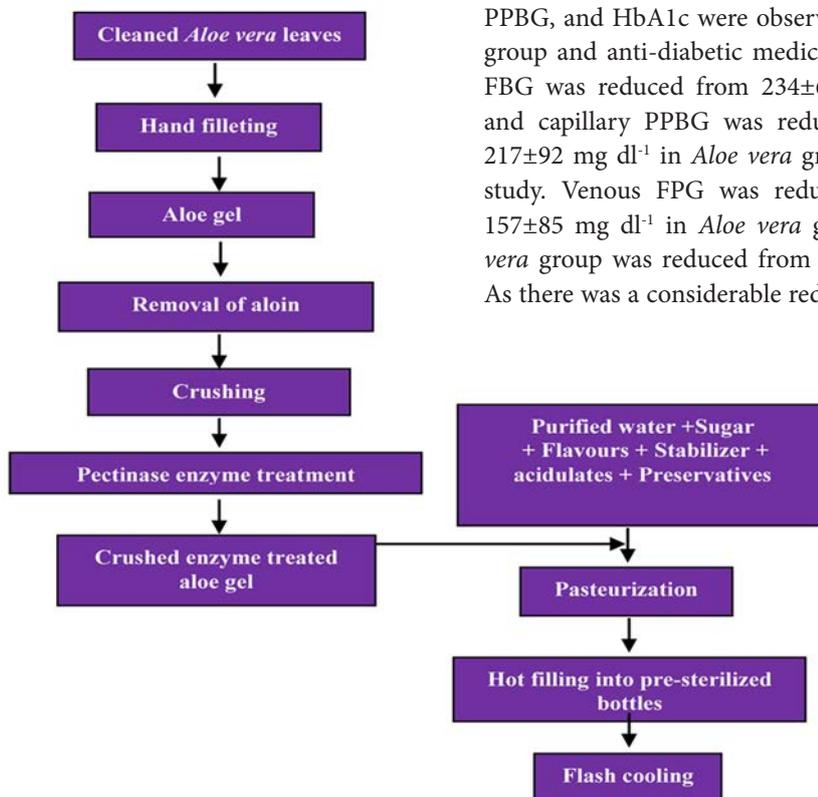


Figure 1. Process flow diagram of *Aloe vera* beverage production



Figure 2. Aloe vera ready-to-serve beverage

oral administration of *Aloe vera* ready-to-serve beverage, anti-diabetic formula of *Aloe vera* beverage could be considered as a food supplement to prevent Type 2 diabetes mellitus.

Many *Aloe vera* RTS beverages have a high acceptance in several countries with the approval of Food and Drug Administration, USA. Processing of the beverage, in this research, has been carried out with precautions to preserve the health beneficial components maximally. The product has been prepared to meet Sri Lankan Standards.

Anti-diabetic effects of *Aloe vera* leaf gel have been reported by several researches. However, research studies related to anti-diabetic effects of aloe based processed food products are few. To the best of our knowledge, this is the first study in Sri Lanka to describe the development of anti-diabetic aloe based RTS and its clinical evaluation. The developed product would benefit the pre-diabetic and diabetic population in Sri Lanka. The potential applications of the product developed would set a novel trend in the beverage industry, by initiating production of both normal formula and anti-diabetic formula of aloe-based RTS beverage. Furthermore, the aloe-based RTS beverage developed can be used as a food supplement, to address the alarming increase of T2DM among the youth and adult population.

Aloe vera is widely cultivated in Colombo and the coastal areas (Mannar, Jaffna, Hambantota, etc.) of the island and can be easily obtained for the preparation of the product.

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Assessing geo-thermal energy development potential in some thermal spring areas of Sri Lanka



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Energy is the main governing factor in the development of societies and economies of the world. Geothermal energy is one of the best sources for generating energy globally. It is large enough to sustain a long-lasting energy supply while being economically and technically accessible and environmentally friendly. Temperature of the earth crust increases with increasing depth at varying rates, depending on the location. The natural temperature gradient is about 25-30 °C km⁻¹ in depth. However, in some regions of the world the temperature gradient is much greater than this natural gradient. Such places are known as geothermal sources and when utilizable, is known as geothermal resources.

In Sri Lanka, geothermal energy is manifested only as thermal springs, which are located in a geographically defined belt extending from Trincomalee to Hambanthota. They appear to be connected with the boundary of the Highland complex and Vijayan complex (Fig. 1). However, use of geothermal energy in Sri Lanka so far, is restricted to bathing and non-organized recreation activities.

This research study attempts to assess the potential of selected thermal springs in Sri Lanka for energy production, or develop these as centers of economic value. The study focuses to identify zones of high subsurface temperature associated with thermal springs and assess its natural obtainable thermal energy for possible extraction for utilization. Thermal springs located in Kapuralla, Mahaoya, Marangala, and Nelumwewa were selected for the study. Existing remote sensing data, such as aerial photographs (1:50,000), satellite images and primary investigation maps, assisted the exploration of geothermal prospects in the areas. A detailed geological mapping programme was carried out during the study covering the areas of the selected thermal springs, at a scale of 1:25,000 to cover 100 km² in each area.

Water samples were collected from the study area to include thermal water, surface water, shallow groundwater (dug well depth < 10 m)

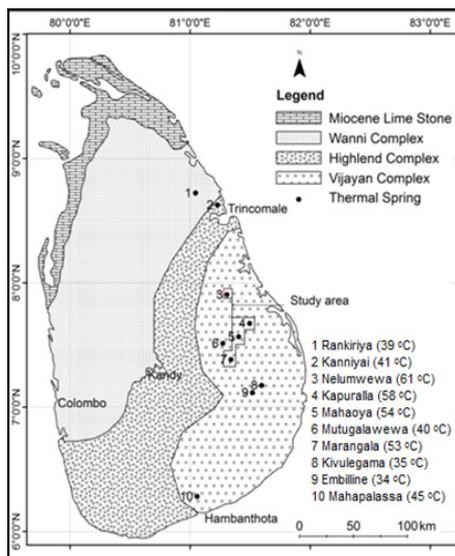


Figure 1. Locations of thermal springs in Sri Lanka (Modified after Disanayake and Jayasena, 1988)

and deep groundwater (tube well depth ~30-40 m). Initial sampling identified the effects of climate on local groundwater and was conducted in two stages; before the monsoon (dry season) and after the monsoon (wet season).

Another series of water samples were collected from pre-defined locations based on geological structures (shear zones, lineaments) to understand the connection between local groundwater and thermal spring water. Thermal spring water was used to estimate the temperature of the geothermal reservoirs, using geothermometers.

Vertical Electrical Soundings (VES) were carried out using Schlumberger array with half the current electrode spacing ranging from 1.5 m to 85 m. In order to maintain a significant potential difference, half potential electrode spacing was increased in steps, from 0.5 to 5 m. The sounding data were analyzed using *IP2Win* computer software and the resistivity and the corresponding thickness of different subsurface layers were calculated. Apparent resistivity contouring was carried out at four selected depths (Fig. 2). “Inverse Distance Weighted” method was used to compare and isolate the distribution of low resistivity zones at depths representing deep extending weathered, fractured zones or thermal water pathways. Furthermore, two-dimensional electrical imaging/tomography surveys were carried out, using forty-two (42) electrodes to obtain an approximate picture of the true subsurface

resistivity distribution and changes in the vertical as well as horizontal direction along the survey line.

The geological, geophysical and geochemical data collected were used to calculate energy content of the geothermal reservoir associated with thermal springs. The calculation was based on the geometry and physical parameters of the geothermal reservoir. A Monte Carlo simulation technique was used with triangular probability distribution to calculate the energy content of a geothermal system according to volumetric approach.

Based on hydrochemistry and isotope signature of the selected thermal springs, they can be described as belonging to non-volcanic low mineralized geothermal systems, while thermal groundwaters are of Na-K, Cl-SO₄ type. The non-thermal shallow groundwater of the area is of non-dominant cation and anion type. Thus, Sri Lankan geothermal systems show characteristics of Hot Dry Rock (HDR) systems. The average reservoir temperature of the four thermal springs according to the Na-K-Mg, Na-K, K-Mg and SiO₂-temperature geothermometry is about 150 °C. Regional geological structures appear to be the dominant controlling factor for the formation of the geothermal fields in Sri Lanka. A noteworthy fact is that, seven out of ten thermal springs investigated in Sri Lanka are situated close to dolerite dykes. Deep circulation of ground water through faults/fracture would mine heat from the dikes and up flow through the faults/fractures. Results of the resistivity investigation suggest that thermal water flow to surface along the fault/fracture or axis of intersection of deep extending two fracture zones in the crystalline rock.

Sri Lankan geothermal systems are water dominated, dynamic and are low to medium enthalpy systems. According to volumetric assessment, it is most likely that the electrical power production capacity will lie between 4.38 and 8.61 MW for Nelumwewa, 460 and 461MW for Mahaoya-Kapuralla and 12.2 and 12.3 MW for Wahawa, if the recoverable heat is used for a period of 30 years. It will be between 2.63 and 5.16 MW for Nelumwewa, 276 and 277 MW for Mahaoya-Kapuralla and 7.37 and 7.39 MW for Wahawa, if it were used for 50 years. Further, it was observed that the average power generation for 30 years is higher than the average power generation for 50 years. The most probable power generation outcome for 30 years is 6.20, 455 and 12.1 MW for Nelumwewa, Mahaoya-Kapuralla and Wahawa 3.72 MW for Nelumwewa, 273 MW for Mahaoya

and Kapuralla and 7.2 MW for Wahawa geothermal system for 50 years. Therefore, accessible economic geothermal resources are limited in Sri Lanka.

Thus, the identified accessible economic geothermal resources are limited in Sri Lanka and the systems are not of high enthalpy type. Yet, these can be used for electrical power generation using binary power generating methods. Direct heat applications are more cost effective, simple and less complicated for utilizing geothermal sources of Sri Lanka.

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HETC Project of the Ministry of Higher Education (Grant No. HETC/W3/PGIS/Activity 2.2)

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Kumara, S. M. P. G. S. (2015). Assessing Geo-Thermal Energy Development Potential in Some Thermal Spring Areas of Sri Lanka, MPhil Thesis, Postgraduate Institute of Science, University of Peradeniya. Pp 113.

Institution where research was carried out

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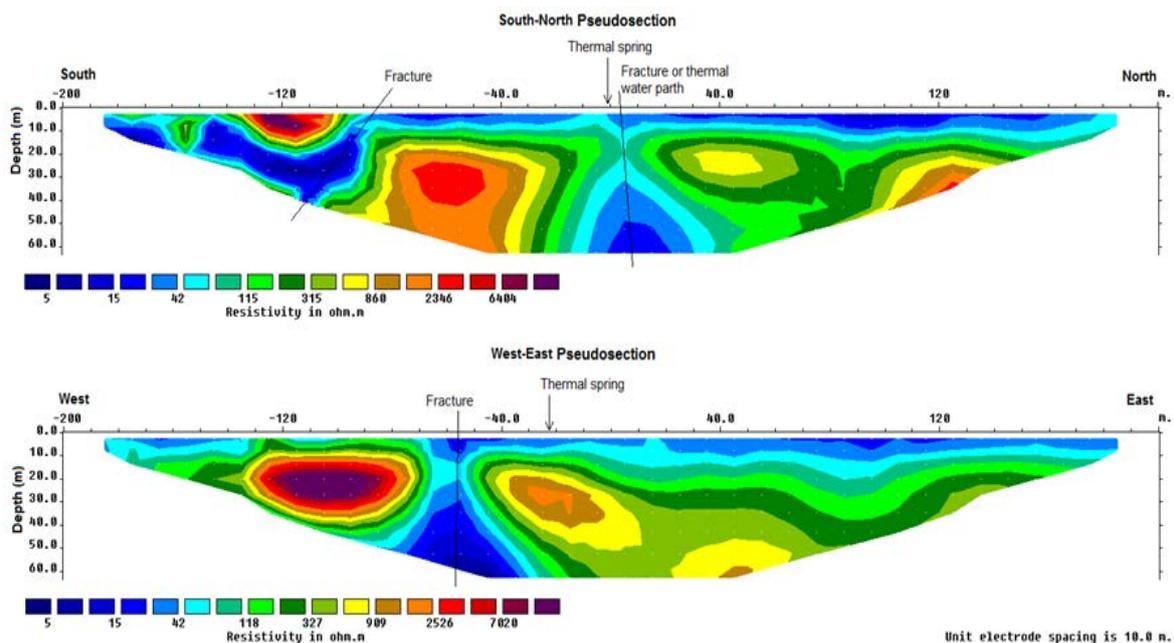
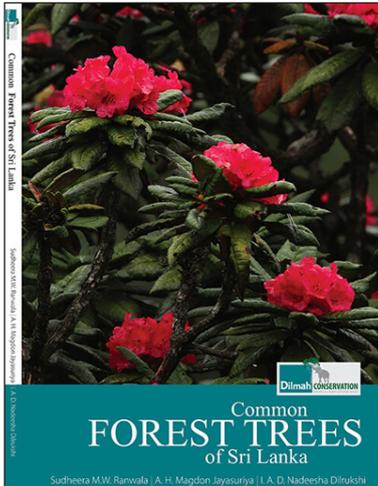


Figure 2. Apparent resistivity pseudosections of Mahaoya (near to the thermal spring)



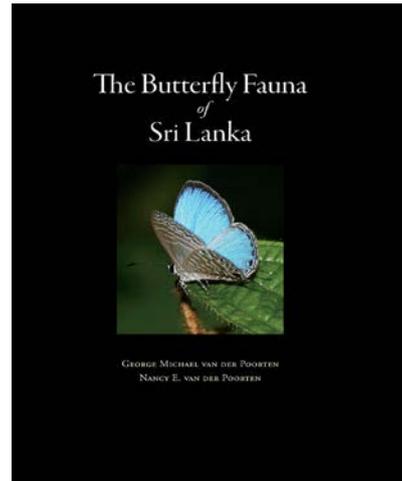
Common Forest Trees of Sri Lanka
Sudheera M. W. Ranwala, Dr. A.H. Magdon Jayasuriya and I.A.D. Nadeesha Dilrukshi
 Published by Dilmah Conservation, Sri Lanka
 ISBN 978-955-0081-22-6
 Price: Rs. 550/-
 Available at Dilmah Tea Centres in Colombo

“Common Forest Trees of Sri Lanka” provides an account of the diverse forest types and the trees therein, a detailed guide, identifying more than 100 common forest trees in Sri Lanka.

The book consists of two main chapters. The first, gives information on forests and trees. The second chapter is an identification guide to common trees of the Dry zone, the Wet Lowland and the Montane forests of Sri Lanka. It carries clear, high resolution colour photographs of 124 forest tree species, their leaves, fruits and flowers. The diagrams of leaves show shape, venation pattern and also gives dimensions. The descriptions help in the correct identification of forest tree species. Further, the conservation status of each tree species is given.

The book also gives additional information such as the tallest and widest tree recorded in Sri Lanka, trees which hold religious and historical significance, protected trees and the tree with the largest inflorescence.

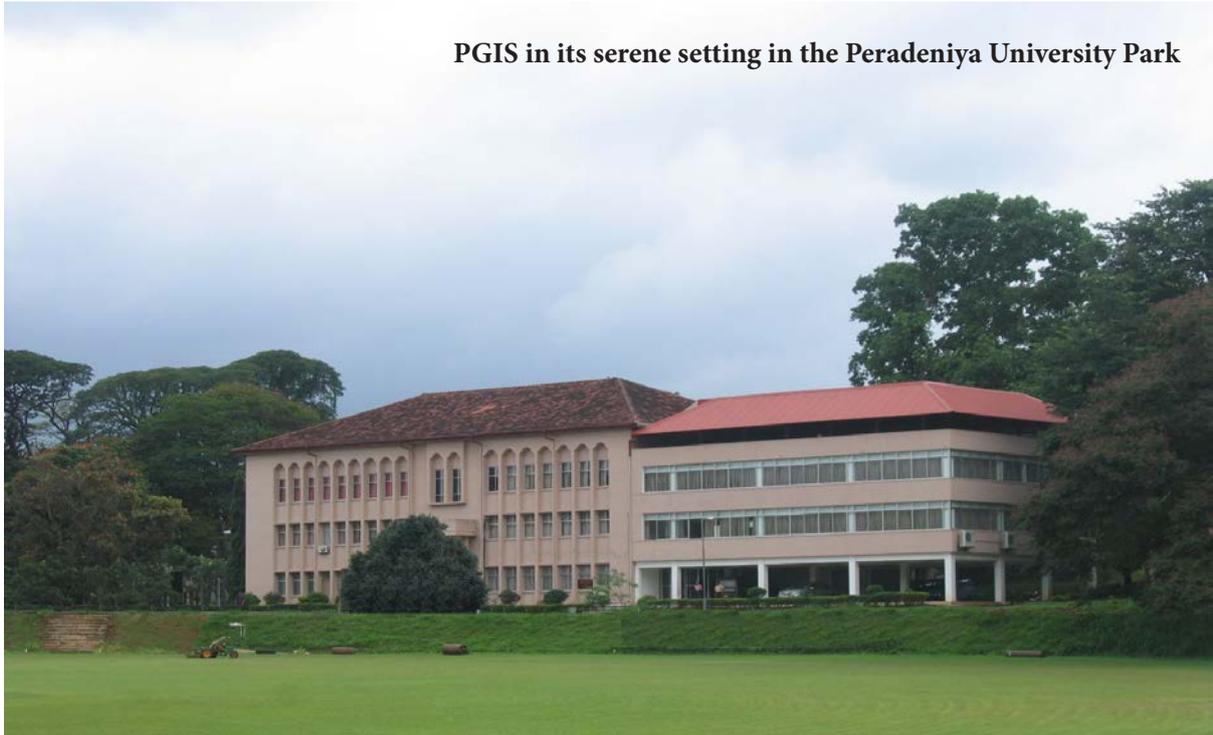
The publication is less technical, making it appeal to the general public—especially for those without a deep knowledge on technical/scientific aspects of the flora. It is also appealing to field botanists as an easy identification guide to common forest trees of Sri Lanka



The Butterfly Fauna of Sri Lanka
George van der Poorten & Nancy van der Poorten
 Published by Lepodon Books, Canada
 ISBN No. 978-1-77136-189-7
 Price –Hardback: Rs.7500.00
 (within Sri Lanka from Lepodon Books)

“The Butterfly Fauna of Sri Lanka” gives a comprehensive account of the lives of all 247 species of butterflies in Sri Lanka, while providing critical information necessary for their conservation. The book comprises ten chapters and the first few chapters are devoted to the history of the study of butterflies in Sri Lanka and their biogeography, classification, morphology, distribution, and conservation. The remaining chapters present the ecology of the butterfly families; Hesperidae, Lycaenidae, Nymphalidae, Pieridae, Papilionidae and Riodinidae. Each butterfly species is addressed in detail with information on identification, similar species, status, distribution, habitat, adult behavior, immature stages, larval food plants, and, for threatened species, conservation concerns. For easy reference, the supplementary material provides lists of species with the common name, scientific name and authorities, endemic status, and distribution by climatic zone, as well as a list of larval food plants and adult nectar sources. Overall, the book is a reference and an essential guide to the butterfly fauna of Sri Lanka and would be of interest to a wide variety of professionals concerned about the natural world and the general reader who has a fascination with these beautiful creatures.

PGIS in its serene setting in the Peradeniya University Park



Vision of the PGIS

To be an internationally renowned Centre of Excellence in Asia for Postgraduate Education and Research in Science

Mission of the PGIS

The mission is to contribute to the development of a knowledge-based society with social sensitivity, ethical rectitude and economic prosperity through postgraduate level education and research. Also, to disseminate knowledge in an intellectually stimulating, efficiently managed, harmonious academic environment.

Scope of “PGIS Postgraduate Research Highlights”

The Postgraduate Institute of Science (PGIS) of the University of Peradeniya that has been in existence since 1996 is celebrating its 20th Anniversary this year. With the establishment of the PGIS, postgraduate research in science based fields received a new lease of life. During the last 20 years, PGIS has grown into a strong robust tree with spreading branches representing the major disciplines in science. It harbours a scientific community from across faculties, embracing scientists in many specialities.

With the 3rd Annual Research Congress of the PGIS, being held this year, the research of postgraduates who received their MPhil and PhD Degrees at the last Convocation, The General Convocation 2015 of the University of Peradeniya, have been brought to life in the form of Postgraduate Research Highlights. The recipients of the postgraduate research degrees are also brought to life with a short personal profile and a photograph. Thus, the unseen young researcher behind the study makes an appearance in this publication.

The PGIS of the University of Peradeniya is privileged to be a national institute giving leadership and guidance not only to the young and upcoming researchers of the University of Peradeniya but also to researchers in other universities, particularly those in newer universities. In an era where research standards are declining, sacrificing quality for quantity, it is timely that postgraduate research output is brought to light for its improvement, applications and future collaborations. The publication, PGIS Postgraduate Research Highlights has been launched to meet these goals.

BOARDS OF STUDY

The PGIS offers postgraduate training in various specialities in science. The research programmes of the PGIS are conducted through ten Boards of Study:

- Biochemistry and Molecular Biology
- Chemical Sciences
- Earth Sciences
- Environmental Science
- Mathematics
- Physics
- Plant Sciences
- Science Education
- Statistics and Computer Science
- Zoological Sciences

In addition to the M.Sc., M.Phil. and Ph.D. programmes, the PGIS routinely conducts workshops, certificate courses and short-term training programmes, and offers consultancy services.

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The PGIS offers postgraduate research degree programmes leading to the Degrees of Master of Philosophy (M.Phil.) and Doctor of Philosophy (Ph.D.). Candidates registered for these degrees are required to engage in a research project under the guidance of a supervisor. The minimum requirement for registration for an M.Phil. or Ph.D. degree is either an M.Sc. degree or a B.Sc. degree in the relevant area of study from a recognized university or any other equivalent qualification. Research programmes are available for M.Phil./Ph.D. candidates under different Boards of Study.

Occasional studentships are available for those who wish to follow courses of choice and use other facilities over short periods without being registered for a postgraduate degree programme.

The PGIS welcomes applications from prospective students for M.Phil./Ph.D. (full-time or part-time) programmes. Please see the PGIS home page (<http://www.pgis.lk>) for more details and application forms.

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